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THE EFFECT OF HYPOGLYCEMIA ON THE ELECTROENCEPHALOGRAM AT VARYING DEGREES OF OXYGENATION OF THE BLOOD

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Accepted for publication November 21, 1941

The interaction of anoxia and hypoglycemia has been the subject of a number of studies (E. Gellhorn and collaborators, McQuarrie and others). It has been shown that the vasomotor center is greatly sensitized to anoxia at low blood sugar levels (Gellhorn, Ingraham and Moldavsky). On the other hand, hypoglycemic convulsions were inhibited rather than enhanced by anoxia (McQuarrie, Gellhorn, Packer and Feldman.) It therefore seemed to be of interest to investigate whether a synergistic action of anoxia and hypoglycemia exists with regard to the somatic nervous system if criteria other than convulsions are chosen as indicators. For this purpose the effect of hypoglycemia on the electroencephalogram was studied at varying degrees of oxygenation of the blood.

The electroencephalogram (E.E.G.) was chosen as an indicator of cortical somatic excitability although it is well known that the cortex contains efferent neurons influencing the activity of autonomic centers at lower levels. However, it is well established that conditions involving a decreased excitability of the cortical somatic centers are paralleled by corresponding changes in the E.E.G.

METHOD. The experiments were performed on anesthetized cats (100 mgm./kgm. chloralose subcutaneously) and on unanesthetized rats. In the former blood pressure was recorded from the carotid artery with the Hg manometer and the brain potentials were led off by means of wick electrodes from the parietal lobe of the brain or from the skull by inserting two screws into the calvarium. In the rat experiments we employed Hoagland's technique and inserted two phonograph needles, insulated except for the tip, into the skull of the rats. For recording the potentials a push-pull amplifier and an Offner ink recorder were used.

The cats inhaled oxygen/nitrogen mixtures from Douglas bags; the rats were placed in suitable bottles through which the desired oxygen/nitrogen mixtures were blown. In order to secure regularly hypoglycemia adreno-demedullated

[1] Aided by a grant from the John and Mary R. Markle Foundation.

rats were used. Five units of insulin[2] per kilogram were injected subcutaneously.

Results 1. *Interaction of anoxia and hypoglycemia.* If rats are allowed to inhale 7 per cent oxygen no conspicuous changes in the E.E.G. are observed when the animals are subjected to this low oxygen tension for an hour or more. If, however, 7 per cent oxygen is administered during insulin hypoglycemia porfound alterations in the E.E.G. occur.

Figure 1 shows that inhalation of 7 per cent oxygen changes the electrical potentials fundamentally by causing very large, slow potentials to appear on which the fast alpha waves are superimposed. On readmission of air a gradual restoration of the original aipha waves is observed. If, then, glucose is injected and the hypoglycemia thereby eliminated the inhalation of 7 per cent oxygen, even for a longer period of time, remains without effect.

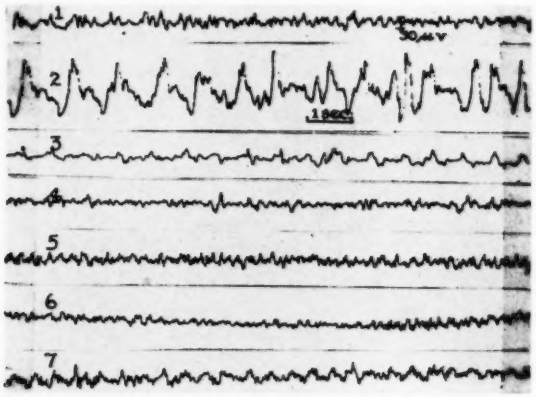

Fig. 1. Adrenalectomized rat. Five U. insulin subcutaneously. 1, 21 min. after injection. 2, 2 min. 7 per cent O_2. 3, after 2 min. in air. 4, after 3 min. in air; blood sugar 43 mgm. per cent; 7 cc. 10 per cent glucose intraperitoneally. 5, 25 min. later (air) 6, 3 min. 7 per cent O_2. 7, 8 min. after air.

It is very interesting to note that the type of effect illustrated in figure 1 is similar to that occurring without the addition of anoxia in insulin hypoglycemia, provided that the blood sugar falls sufficiently low and this condition is maintained for an adequate length of time. This and similar observations suggest that anoxia and hypoglycemia act synergistically on the cortical potentials.

Whereas figure 1 illustrates the action of anoxia on the brain potentials at a relatively mild hypoglycemic state the effects resulting from the interaction of anoxia and hypoglycemia when the blood sugar has fallen to a very low level (30 mgm. per cent) are slightly different. Under these conditions the alpha waves are greatly diminished in amplitude and slow delta waves appear. On administration of 7 per cent oxygen both alpha and delta waves are diminished so that the brain potentials disappear almost completely. On readmission of air brain waves similar to those obtained prior to the administration of 7 per cent oxygen are gradually restored.

[2] Kindly supplied by Eli Lilly & Company.

Figure 2 shows a record from an experiment on an anesthetized cat in which not only the effect of anoxia at various blood sugar levels on the E.E.G. but also on the blood pressure is recorded. Although hypoglycemia does not alter the E.E.G. of the cat in chloralose anesthesia to as great a degree as seen in the unanesthetized rat the reaction to anoxia is basically the same. In the anesthetized cat, too, it is seen that anoxia and hypoglycemia act synergistically on the brain potentials and that the effect on the E.E.G. resulting from anoxia increases progressively with falling blood sugar. No effects are seen after inhalation of 8.1 per cent oxygen at a blood sugar level of 108 mgm. per cent,

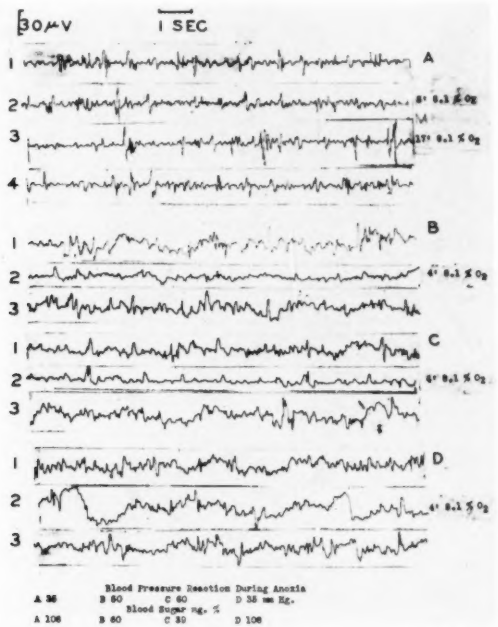

Fig. 2. Cat, 100 mgm. chloralose per kilogram subcutaneously; 5 U. insulin. First and last record in A, B, C and D is obtained while the animal is inhaling air. Second and third in A and second in B, C and D were obtained during inhalation 8.1 per cent O_2.

Blood pressure reaction during anoxia in mm. Hg: A, 35; B, 50; C, 60; D, 35.

Blood sugar, mgm. per cent: A, 108; B, 60; C, 39; D, 108.

even after seventeen minutes, whereas at 60 mgm. per cent and still more at 39 mgm. per cent blood sugar a profound diminution of the potentials occurs. The fundamental difference in the reactivity of the autonomic and somatic systems is well illustrated in this experiment. The blood pressure rises in response to anoxia to an increasing degree with progressing hypoglycemia whereas the cortical potentials are diminished progressively under the same conditions. The synergism of anoxia and hypoglycemia is present for both autonomic and somatic nervous systems, but whereas the somatic system suffers progressively in its

excitability under these conditions, the autonomic nervous system shows a greatly increased reactivity.

2. *The effect of inhalation of pure oxygen on the E.E.G. under conditions of hypoglycemia.* The fact illustrated in the preceding paragraphs, that the effects of low oxygen on the E.E.G. are greatly enhanced under conditions of hypoglycemia, made it not improbable that the effects of hypoglycemia may be alleviated to a certain extent by improving the degree of oxygen saturation of the blood and consequently of the brain. We therefore attempted to show that the effects of hypoglycemia on the E.E.G. could be diminished by the inhalation of pure oxygen. These experiments were carried out on unanesthetized rats exclusively. Although the inhalation of 100 per cent oxygen has no effect on the E.E.G. of a normal rat, it produced marked changes under conditions of insulin hypoglycemia.

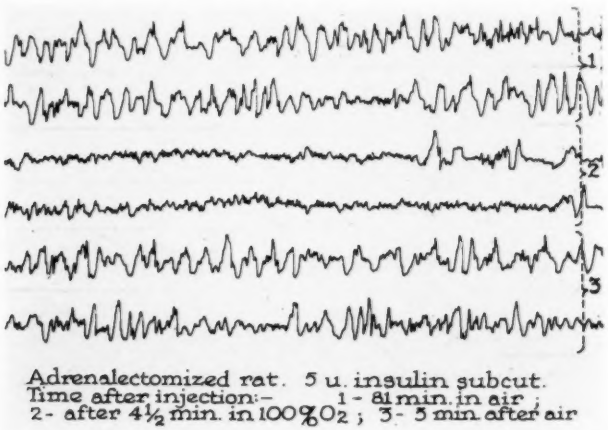

Fig. 3. Adrenalectomized rat. Five U. insulin subcutaneously. Time after injection: 1, 81 min. in air. 2, after 4½ min. in 100 per cent O_2; 3, 5 min. after air.

Figure 3 shows a record in which the injection of insulin had produced large delta waves on which the more frequent alpha waves were superimposed. This typical effect of hypoglycemia occurred 81 minutes after the injection of insulin into the adreno-demedullated rat. When the animal was allowed to inhale oxygen and a record was taken after 4½ minutes of oxygen inhalation, the delta waves had largely disappeared and a record was obtained similar to that found prior to the injection of insulin. If, however, oxygen was no longer administered and the animal inhaled air the delta waves reappeared and the record was practically identical with that obtained prior to the administration of pure oxygen.

It is frequently found that the E.E.G. during hypoglycemia consists of periods of delta waves with alpha potentials superimposed on them alternating with periods of alpha potentials exclusively. Also in this case the inhalation of 100 per cent oxygen abolished the delta potentials thereby restoring an E.E.G. of pure alpha potentials as existed before the administration of insulin.

The experiments show clearly that the effects of hypoglycemia can be completely offset by the inhalation of pure oxygen. It is, however, important to note that this statement does not apply for all conditions of hypoglycemia. If the hypoglycemia is so severe and prolonged as to lead to an almost complete disappearance of brain potentials (prior to the convulsive state) no restoration of normal brain potentials results from the administration of oxygen under these conditions, although brain potentials are usually restored by the injection of sugar.

Discussion. In several investigations on anoxia (Cortell, Carlson, Greenberg, Lambert and Gellhorn), it was shown that whereas the somatic nervous system is depressed during anoxia, the sympathetic system is in a state of greater tonicity and excitability. It is interesting to note that this difference in the reactivity existing between the somatic and autonomic nervous system is preserved under conditions involving anoxia and hypoglycemia at the same time. It was shown by the fact that hypoglycemia and anoxia depressed cortical potentials more than corresponds to the algebraic summation of the effects produced by anoxia and hypoglycemia when studied separately. A similar synergism exists with regard to the autonomic nervous system. When the reactivity of the vasomotor center is studied it is seen in a greatly increased rise in blood pressure under conditions of anoxia and hypoglycemia. The synergism seen on the autonomic nervous system may be interpreted as an attempt to offset the synergism acting on the somatic nervous system for the purposes of homeostasis.

Our experiments give further support to the assumption that the rate of oxidation of the brain depends on the sugar level as well as on the oxygen tension of the blood (cf. Gellhorn, Ingraham and Moldavsky concerning the older literature). This assumption makes it understandable that the effects of anoxia on the E.E.G. are aggravated by the hypoglycemia, and the changes in the brain potentials induced by hypoglycemia can be offset by the inhalation of 100 per cent oxygen. There are, however, definite limits to the possibility of substituting glucose for oxygen. Although injection of glucose restores brain function when its activity is almost completely lost due to hypoglycemic coma, no such effects are obtained with pure oxygen inhalation; but the latter procedure is effective as long as the hypoglycemia has not proceeded further than to the state in which the brain potentials are characterized by the partial or exclusive occurrence of large delta waves. Moreover, no clear evidence could be found that an increase in blood sugar above the normal level resulting from injection of glucose would increase the resistance of the brain to anoxia.

The observations reported in this paper support the interpretation which McFarland and Forbes give to their work on the effect of hypoglycemia and anoxia on visual dark adaptation, namely, that the synergistic effects of anoxia and hypoglycemia as well as the antagonistic effects of pure oxygen on the effect of hypoglycemia are not due to their action on the visual purple but on that part of the central nervous system which is involved in vision.

The experiments cannot be explained on the basis of alterations in the blood sugar induced by the inhalation of 7 per cent oxygen since in order to produce a significant lowering of the blood sugar level in adrenalectomized rats two hours of anoxia are required but not 2 to 3 minutes as used in the present experiments. Moreover, the synergism of anoxia and hypoglycemia on brain potentials was present in adrenalectomized as well as adrenalectomized-vagotomized animals although even prolonged anoxia failed to alter the blood sugar in the latter group (Feldman, Cortell and Gellhorn).

SUMMARY

The interaction of hypoglycemia and anoxia, and the effect of the inhalation of pure oxygen during insulin hypoglycemia was studied in anesthetized cats and unanesthetized rats with regard to brain potentials. It was found that: 1, the effect of anoxia on brain potentials is greatly aggravated during insulin hypoglycemia; 2, the action of hypoglycemia on the brain potentials can be offset by the inhalation of pure oxygen.

The experiments support the assumption that the excitability of the cortex in vivo depends on both oxygen tension and blood sugar, and that both factors are involved in the rate of oxidations of the brain.

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VARIABILITY OF THE HERING-BREUER REFLEXES IN THE DOG UNDER SODIUM EVIPAL ANESTHESIA[1]

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The changes in the character of the respiratory act associated with inflation and deflation of the lungs are considered as being due to the interaction of inhibition and excitation. The specific inhibitory as well as excitatory functions of the pulmonary afferent impulses have been generally accepted though variously interpreted. (For a concise review of past and current concepts regarding the Hering-Breuer phenomena, see (2) or (3).)

Gross alterations in the Hering-Breuer reflexes associated with changes in depth of sodium evipal anesthesia have been observed and are considered pertinent to a better understanding of these phenomena. The purpose of this paper is to illustrate these changes and their significance.

METHODS. The experimental methods are the same as previously described (5). Inflation and deflation of the lungs were carried out with the chest wall intact, by increasing and decreasing the air pressure within the respiration system to which the animals were connected. All pressures employed were 70 mm. of water above or below atmospheric pressure. Seventy millimeters water pressure above atmospheric is described as "positive" or inflationary; 70 mm. water below atmospheric pressure is for convenience described as "negative" or deflationary. This study is based upon experiments upon 21 dogs.

RESULTS. Figure 1: a reduction in lung volume (neg. press.) in this lightly anesthetized animal induced an initial quickening of breathing (the typical response) that lasted for seven breaths. The rate then became less than the antecedent normal, probably due to a decrease in central chemical drive (CO_2) (5). After the anesthesia was deepened slightly (D) the same deflation produced an identical initial acceleration which did not diminish appreciably. Under relatively deep anesthesia (E) the effectiveness of pulmonary deflation in speeding breathing was great.

Under light anesthesia a transient slowing of respiration followed lung inflation. The breathing during the inflation was characterized by regular, rapid, forceful expirations; these forceful expirations were much smaller and intermittent before inflation.

After deepening the anesthesia (C) the active expirations are brought on by

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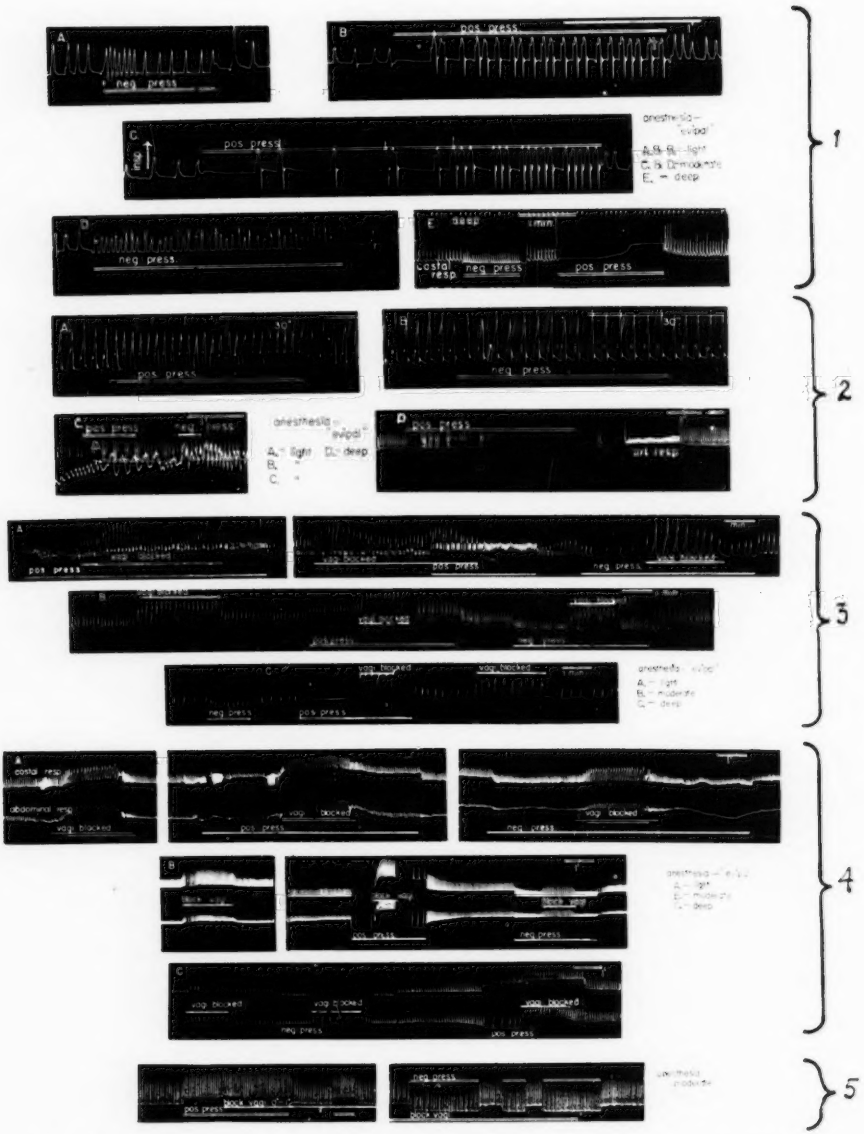
[2] National Research Council Fellow.



Figs. 1, 2, 3, 4 and 5. Read from left to right. An upstroke indicates inspiration. *Pos. press.*, *neg. press.* indicate respectively inflation and deflation pressures of 70 mm. H_2O. greater or less, respectively, than atmospheric pressure.

inflation although entirely absent under normal lung volume. The slowing phase of inflation is much longer, paralleling decreasing sensitivity to carbon

dioxide, and the terminal acceleration is much more in evidence. The rate in this latter phase is greater than that which obtains following removal of the positive pressure (C).

Figure 2 illustrates a type response to inflation and deflation frequently seen under *light* evipal (and pentothal) anesthesia. Lung inflation augments breathing by shortening the duration of inspiration and expiration; lung deflation slows it by lengthening both phases. After the anesthesia was deepened (C and D) lung inflation (pos. press.) which previously shortened expiration now prolonged it.

The responses of the animal shown in figure 3 are somewhat comparable to those of figure 2. Inflation accelerates breathing and deflation slows it. Vagal block during inflation (A) does not slow breathing as much as it does when lung volume is normal; vagal block during negative pressure is associated with a greater increase in amplitude and a slower rate than obtains with normal and greater than normal lung volumes. After more sodium evipal is given (B) the speeding with inflation is less and deflation augments breathing instead of slowing it. The differences in reaction to vagal cold block observed under light anesthesia with changing lung volume are no longer apparent. The administration of more anesthetic changes the inflation response to a slowing of breathing that is abolished by vagal block; vagal block formerly merely decreased the acceleration. Deflation still speeds respiration.

Another type variation is illustrated in figure 4. When very lightly anesthetized the animal reacted to lung inflation and deflation by a minor slowing and a fair augmentation of breathing. Vagal block during inflation produced further slowing and abolished the speeding of deflation. The short periods of initial and terminal stimulation are most likely of general somatic afferent origin; the stimuli are provided by the starting and stopping of the experimental procedures. Under moderate anesthesia (B) the rate of breathing with normal innervation and with the vagi blocked was faster than the breathing under similar circumstances with lighter anesthesia (A). Pulmonary inflation produced a prolonged powerful expiration especially abdominally (B); blocking the vagi did away with the powerful expiratory effort shown by the rapid inspiratory progression of both costal and abdominal records which immediately followed the application of the blocks. Deblocking reinstituted the expiratory effort. Vagal block during the fast breathing of decreased lung volume was associated with a slower breathing than was present during block under normal and supernormal lung volumes. The same relationship of rates under block during normal and decreased lung volume are noted in (C) under deep anesthesia. The rate under block and inflation in (C) was greater than that during block with lung volume normal. The slowing and the increased strength of expiration that attends inflation was less when the anesthesia was deep than when moderate.

Figure 5 illustrates an atypical reaction to inflation and deflation that was unchanged by block and did not become typical at any depth of anesthesia short of death.

Discussion. While certain variants are presented in the figures during light

anesthesia, the reactions of *deeply* anesthetized animals to pulmonary inflation and deflation are almost invariably qualitatively similar and are of this type: inflation slows and deflation speeds breathing. Under light sodium evipal anesthesia great variability is noted. If the reactions typically found under deep anesthesia are present under light anesthesia they are of lesser degree and are less well maintained than under deeper anesthesia. Compare A and B with E of figure 1, and A with B and C of figure 4. Frequently lung inflation speeds and deflation slows breathing with light anesthesia and yet produces the usual responses with deep. Compare A and B with C and D of figure 2, and A with C of figure 3. Occasionally when light the animal will initially react with the typical deep anesthesia response and shift during the procedure to the opposite reaction type as in A, B and C, of figure 1. Those animals that react to an increased and decreased lung volume with an increased and decreased rate of breathing respectively fall into two main groups: those in which vagal block diminished the response, figure 3 A, and those in which it does not, figure 5. The reaction of the former group is therefore at least in part due to pulmonary vagal afferent stimulation and that of the latter mainly if not entirely due to extra vagal stimuli. The source of the extravagal excitation has not been determined; it is presumed that it lies in the respiratory muscle and joint proprioceptors (1, 6). Increasing the depth of anesthesia obliterated the countertypical reactions in five cases out of the six observed; the one exception is the experiment from which figure 5 was taken. Figures 2 and 3 illustrate the transition effected in the major group.

The above variations of response and their transitions cannot be tentatively explained upon the main premise that a changing inhibition of breathing is the predominant action of the vago-pulmonary afferent simulation associated with lung volume change. However, if one should employ as a main premise the tenets of pulmonary vagal reflex action held by Gesell (3), a logical explanation may be constructed very simply. In Gesell's schema afferent impulses of pulmonary-vagal origin are predominantly excitatory and inhibition is considered as a reciprocal function of the primary respiratory motor neurones and not as an attribute of the vagal afferent system. Whether the stimuli of pulmonary origin will be reflected as increased inspiratory or expiratory activity will depend upon the precedence that the stimulus is able to command. Precedence is determined largely by the strength and character of the stimulus, the peculiarities of the central connections of the afferent pathway over which the impulses are propagated, and the balance of the prevailing inspiratory and expiratory drives before arrival of the stimulus.

In these experiments the strength of stimulus is assumed to be constant throughout a single experiment, a constant inflating and deflating force being used. The peculiarities of the central connections of the vagal afferent fibers stimulated are also considered as a constant in any one animal upon anatomical grounds. Impulses set off by inflation stimulate inspiration and expiration, expiration to the greatest extent. The fibers carrying these impulses are therefore thought to have more abundant connections with the expiratory center.

The converse relationship holds for the deflation reflex; thus its receptors may be considered to have more numerous inspiratory central connections. Therefore the variations in the Hering-Breuer reflexes that follow changes in the depth of anesthesia are most likely in greatest part due to change in the balance of the prevailing drives prior to and during the stimulation.

One major change in respiratory drive is now known to take place as the anesthesia produced by sodium evipal is deepened; the central chemical drive, as measured by the animal's response to a fixed increase in the carbon dioxide of the inspired air, is very much more rapidly and profoundly depressed than are the reflex drives, chemical and proprioceptive (5). Since increased central acidity (CO_2) is predominantly an inspiratory drive (3) increasing the depth of the sodium evipal anesthesia will increase the apparent effectiveness of any reflex expiratory drive such as the pulmonary vagal inflation reflex. This is considered to be the probable reason for the change in reaction to pulmonary inflation and deflation that takes place in figures 1 and 4 as the anesthesia is deepened.

Another disturbance in drive balance depends upon the manner of producing the pulmonary vagal stimulation. In this series of experiments, since the chest wall is intact, inflation and deflation of the lungs stimulate both vagal and extravagal afferent systems. The effect upon breathing produced by this extravagal stimulation is, in this series of experiments, opposite to that seen on solely inflating and deflating the lungs; the expansion of the chest and the decreasing diaphragmatic tension attending lung inflation increases the frequency of breathing, and deflation of the chest and increasing diaphragmatic tension slows breathing. Figure 5 illustrates practically a total "extravagal reflex" action in that vagal block has very little effect upon the reactions to positive and negative pressure. Individual variations in the balance between the extravagal and pulmonary vagal proprioceptive drives are seemingly more or less responsible for the differences in reaction to lung volume change at a given level of anesthesia. For example: if the extravagal and pulmonary vagal drives produced by a lung volume change should be equal then the visible effect upon breathing will be slight if any as in A of figure 4; if the extravagal drive is the stronger then the rate of breathing would increase with lung expansion and slow with deflation, figures 2 A, B, 3 A, and 5; should the vagal drive exceed the extravagal the classical Hering-Breuer reflex responses would be obtained as in A, figure 1. If deepening the anesthesia depresses the extravagal drive more than the vagal then an animal having counterclassical reactions to lung volume changes when light might have classical ones when deep. This evidently accounts in part for the shifts as previously described for figures 2 and 3. This is especially evident in figure 3. If the diminution in central chemical inspiratory drive that attends deepening anesthesia was entirely responsible for the changes in reaction to deflation and inflation in this experiment then there would have been no reversal of the deflation reflex response for the pulmonary vagal deflation drive is predominantly inspiratory and is therefore theoretically additive to the chemical drive. Therefore the slowing of breathing associated with a decreased lung

volume (neg. press.) that was accentuated by vagal block (A) must have been due to an extravagal expiratory reflex drive that was greater than the combined existing and building chemical inspiratory and the induced vagal inspiratory drives. Deepening the anesthesia evidently effected a much greater reduction of the extravagal drive than it did the vagal, inasmuch as the deflation response became classical at a moderately deep level, part B. It cannot be assumed that no depression of the pulmonary vagal drive takes place during deepening anesthesia as both the inflation and deflation drives are often definitely depressed as illustrated in figure 4. Compare B and C.

The ease with which these seemingly contradictory experiments can be reduced to a single simple thesis constitutes additional support of Gesell's concept of reflexogenic respiratory control.

SUMMARY AND CONCLUSIONS

Dogs *lightly* anesthetized with sodium evipal show great variability in their respiratory reactions to inflation and deflation of the lungs. When they are *deeply* anesthetized their reactions to pulmonary inflation and deflation are with but rare exception qualitatively similar: inflation slows and deflation speeds breathing. The change from variable to comparable responses is considered to be largely due to the more powerful depression effected by evipal upon the central chemical respiratory drive than upon the reflex drives; and to the greater depressant action of this agent upon the extravagal proprioceptive than upon the vago-pulmonary reflexes.

The changes in character and efficacy of the vagopulmonary respiratory reflexes that attend variations in the depth of sodium evipal anesthesia make it impossible to define upon the basis of evidence obtained from animals anesthetized with sodium evipal the relative rôles that these reflexes play in the control and maintenance of normal breathing.

Of all the concepts regarding the functional characteristics of the Hering-Breuer reflexes that recently formulated by Gesell lends itself most readily and with fewer assumptions to an explanation of the changes that these reflexes undergo when the depth of sodium evipal anesthesia is varied.

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CENTRAL STIMULATION OF RESPIRATION DURING HYPOXIA[1,2]

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Following the discovery of the peripheral vascular proprioceptive and chemoreceptive respiratory reflex mechanism (5), it appeared for a time that the chemoreceptor function of the respiratory center was to be shifted from the central nervous system to the specialized vascular structures. With but a few exceptions (6) (7) (8) (9) (27) it has been the finding that in the absence of the peripheral chemoreceptor mechanisms the anesthetized animal is incapable of making a satisfactory respiratory or circulatory adjustment to lowered partial pressures of oxygen in the inspired air (5) (21) (20) (2) (26) (28) (29) (15) (18) (4) (24) (10) (14).

Quite by chance, during the course of an investigation undertaken to attempt a comparative evaluation of the effects of various commonly used anesthetics upon the respiratory drive mechanisms, it was observed that a few animals under moderately light sodium evipal anesthesia, in which the cervical vagi had been severed and the carotid regions denervated, tolerated lowered alveolar oxygen tensions remarkably well; and in one experiment there appeared a fair hypoxic hyperpnea. This response was entirely abolished by a very slight increase in the depth of anesthesia. This led us to conduct six experiments (4 evipal; 1 pentothal; 1 cyclopropane) *for the purpose of determining whether or not the central hypoxic stimulation could be obtained with regularity* when special effort was expended to maintain the animal at the lightest level of anesthesia compatible with sound physiologic practice.

METHODS. Healthy, mongrel dogs weighing between 7.5 and 14 kgm. were anesthetized with sodium evipal (50 mgm. per kilo—10 per cent aqueous solution), with sodium pentothal (25 mgm. per kilo—5 per cent aqueous solution), administered intravenously, and with cyclopropane by inhalation. The general experimental methods have been described elsewhere (1).

The depth of anesthesia was objectively followed by recording the ipsilateral reflex contraction of the left semitendinosus muscle on stimulating the central end of the cut sciatic nerve, and also by noting the condition of the lid and corneal reflexes.

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[2] Preliminary report Proc. Soc. Exper. Biol. and Med. **133:** P392 (1941).

[3] National Research Council Fellow.

The vagosympathetic trunks were cleanly cut with a very sharp, thin knife low in the cervical region. The carotids were denervated by double ligation and transection of Hering's bundle including the occipital artery. The common, internal, and external carotid arteries were stripped of their adventitia for a distance of at least two centimeters from the bifurcation. Absorbent cotton well saturated with one per cent procaine hydrochloride was then placed over the denuded bifurcation area.

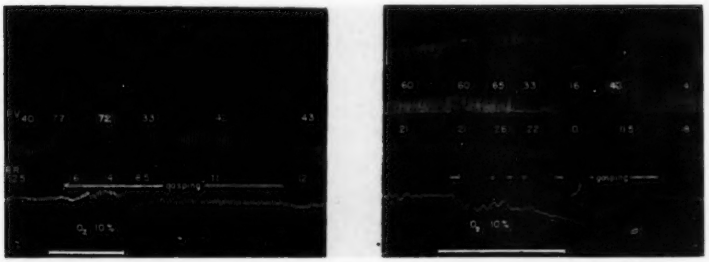
Results. The experiment previously mentioned is shown in figure 1, section 1. Ten per cent oxygen when given after vagal section during light anesthesia resulted in the hyperpnea shown in figure 1, section 1, A. Between A and B the carotids were denervated. No sodium evipal was given during the operation and consequently the anesthesia is lighter after carotid denervation. The rate of breathing had increased and respiration had lost the characteristics ordinarily seen after bilateral cervical vagotomy. The reaction to the same low oxygen mixture (10 per cent) differed from that previously obtained. Periodicity, a decreased tidal air, and an acceleration of rate which was capable of increasing pulmonary ventilation for a short time now appear. The gasping movements which were continuous during hypoxia when the carotid receptors were intact (A) were now intermittent and limited to the periods of greatest respiratory and vasomotor activity. They became continuous for the period of maximal amplitude after the hypoxia was terminated.

Experiment 38 (fig. 1, sec. 2) provides a more comprehensive picture of central hypoxic respiratory stimulation. The reaction of the fully innervated animal to hypoxia is shown in A.

Between A and B the cervical vagosympathetic trunks were severed and the carotids denervated. These procedures necessitated the administration of one cubic centimeter of sodium evipal. The administration of 8 per cent oxygen (B) provoked a fall in blood pressure and a diminution in breathing. Following the hypoxic period a definite increase in pulmonary ventilation occurred which exceeded the pulmonary exchange before the hypoxia. After a thirty minute wait the anesthesia had become almost as light as it was in A. Within the second minute of the low oxygen administration, C, respiratory stimulation began and rapidly increased. Shortly after the termination of the hypoxia a relative apnea occurred. However, one minute after the hypoxic period a respiratory minute volume of 26.4 liters was observed; this was greater than that seen at any time during the hypoxia and differed from the hypoxic hyperpnea in that amplitude played a greater rôle than formerly in its production.

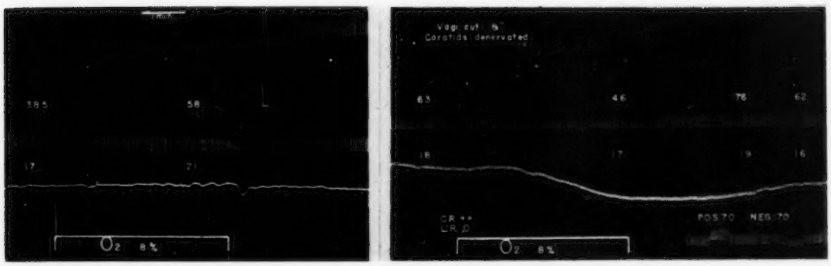
Following this stimulation the carotid denervation was checked and found to be complete and more procaine was applied about the bifurcation. The vagi were also inspected and both found to be completely severed. The low oxygen administration was then repeated twice with the same result as shown in C. Eight-tenths of a cubic centimeter of sodium evipal was then given intravenously; breathing typical of vagotomy was established, and hypoxia evoked only a very transient stimulation and death.

Figure 2 shows the typical hypoxic reaction of the lightly anesthetized animal.

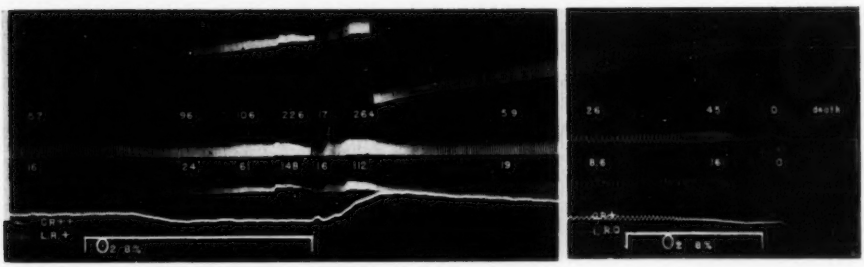


A B

Section 1



A B



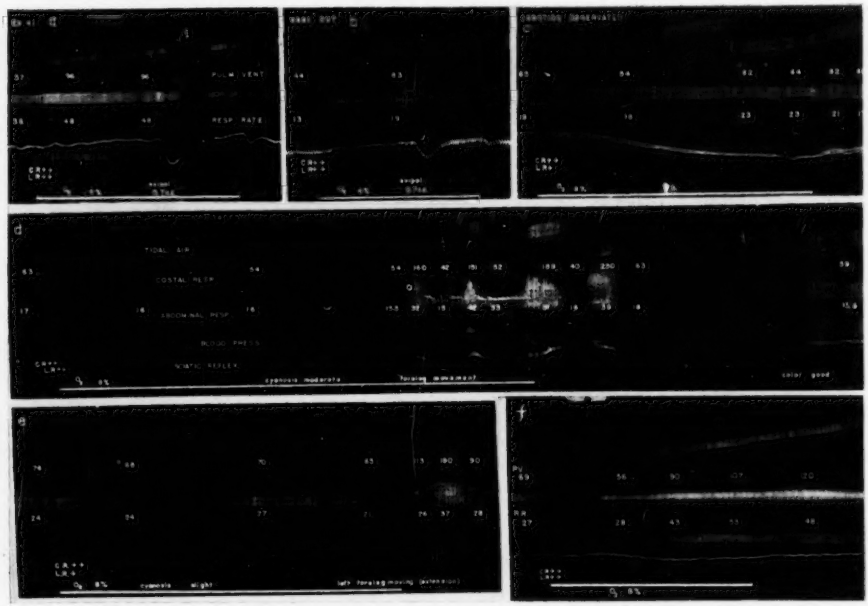
C D

Section 2

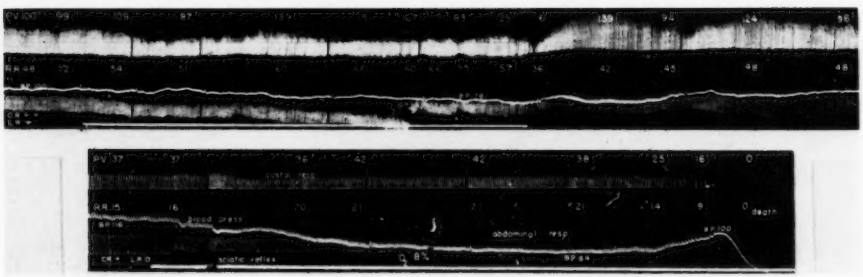
Fig. 1. The tracings from above downward indicate: Time in 5 sec. intervals with minutes shown; the tidal air respiratory excursion; *P.V.*, indicates pulmonary ventilation in terms of minute volume, expressed in deciliters; *C*, costal respiratory excursion; *R.R.*, respiratory rate; blood pressure; abdominal respiratory excursions; fine line shows sciatic reflex indicator, broad line indicates the interval during which special agents were administered; *C.R.*, corneal reflex; *L.R.*, lid reflex; signal marker. In the records of respiration an upstroke corresponds to inspiration; read from left to right. Except as specified the animal breathed room air. Section 1, vagi cut before A. Carotids denervated between A and B. Section 2, vagi cut and carotids denervated between A and B.

One and one half cubic centimeters of sodium evipal had to be given in order to cut the vagi and denervate the carotids; the anesthesia is, therefore, deeper in C than in A. The animal was then subjected to 8 per cent oxygen for nine minutes.

The response to hypoxia after denervation (C) was primarily one of depression. A short postanoxic hyperpnea was evident.



Section 1



Section 2. A, above: B, below

Fig. 2, Section 1. Details are as for figure 1, except denervations occurred as shown. Section 2, vagi cut and carotids denervated before tracings were made: A, light anesthesia; B, deep anesthesia.

The level of anesthesia was then allowed to lighten until it reached that present in A; low oxygen was again administered, D. During the eleventh minute of hypoxia a cyclic stimulation of breathing suddenly began. Coincident with

the periods of hyperpnea, blood pressure rose slightly, and running movements of the forelegs appeared; extreme jactitation was also present. Room air was given to the animal during the third period of hypoxic hyperpnea; all convulsive movements quickly ceased and respiration gradually slowed down. This was followed by a postanoxic hyperpnea that exceeded the hypoxic hyperpnea. It was unattended by general somatic muscular movement.

Following the injection of one cubic centimeter of sodium evipal, hypoxia was attended by the reactions shown in E. The oxygen deficiency was terminated after eleven and one-half minutes without a significant sign of stimulation. However, forty seconds after room air was given, definite signs of respiratory stimulation appeared. Pulmonary ventilation rose to a level which represented a 10.6 liter increase over normal.

The animal was then rested for twenty minutes after which low oxygen was given (F). After a two minute latent period a smooth stimulation of respiration attended the hypoxia; jactitation and somatic movements were *not* present. The rate increase was now a more prominent factor of the hyperpnea than at any time previously. The postanoxic hyperpnea is also evident and follows the hypoxic stimulation without sign of the preliminary depression. Two and one-half cubic centimeters of sodium evipal were then given and hypoxia then was attended by respiratory failure without any sign of stimulation (not illustrated).

One experiment was done under sodium pentothal anesthesia (fig. 2, sec. 2). All the records show hypoxic responses of the fully denervated animal. A demonstrates the rapidly augmenting character of inspiration after vagotomy under very light anesthesia; B shows the change to the slowly augmenting inspiration typical of vagotomy produced by only 0.7 cc. of 5 per cent sodium pentothal. The changes in the hypoxic reaction under sodium pentothal anesthesia as it is deepened appear to be in general the same as those described when sodium evipal is used as the anesthetic agent.

The one experiment performed under cyclopropane anesthesia had a result very like that under evipal and pentothal. The acutely denervated animal when anesthetized with 20 per cent cyclopropane showed great respiratory stimulation in response to oxygen deficiency; this was associated with generalized clonic convulsions. After deepening the anesthesia (cyclopropane, 30 per cent) oxygen deficiency evoked respiratory depression.

Discussion. The hypoxic stimulation of breathing in the acutely denervated, lightly anesthetized animal differs from that of the normally innervated one under similar experimental conditions in that the latent period is prolonged and periodicity is more prominent. The postanoxic hyperpnea is a definite part of the picture in the denervated animal's hypoxic response, whereas it is only rarely seen in that of the normally innervated one.

During the latent period the respiratory changes of blood and tissues must be in a measure qualitatively comparable to those described by Gesell and associates (12) (13) as occurring in dogs subjected to low oxygen tensions while the animal's ventilation was kept uniform (controlled ventilation). Brassfield (3)

has recently shown that the $C^{(H^+)}$ of the brain surface rises under like experimental conditions. It may be inferred from the experiments described here that biochemical changes of a similar nature very likely occur before the actual hypoxic hyperpnea begins, and that an increasing acidity of the cells of the center is probably the direct stimulus responsible for the increased respiratory activity coincident with the subjection of lightly anesthetized, acutely denervated dogs to decreased oxygen tension.

In order to obtain any significant stimulation of respiration by hypoxia in the denervated animal it is necessary to have the animal very lightly anesthetized. It is evident that only a slight increase in the depth of anesthesia will greatly reduce the ability of a denervated animal to make adjustments to the same degree of oxygen lack.

The level of anesthesia at which no, or only a slight, stimulation of breathing attends hypoxia in the denervated animal is in some cases too light to allow surgery to be performed. It therefore appears that the sensitivity of the center to (H^+) ions is very substantially lowered by a small amount of sodium evipal. The mechanism whereby evipal effects a very great reduction of the central stimulating action of (H^+) ions may be closely allied to, or identical with, its depressant action upon brain respiration in vitro. See Jowett and Quastel (16), and Quastel (19).

The postanoxic stimulation of respiration associated with the central hypoxic reaction seems to be pertinent to the question of the relative rôles played by $C^{(H^+)}$ changes, and decreased oxidations (17) within the center in the chemically denervated animal. The postanoxic hyperpnea, which in all cases is greater than that present during hypoxia, likely is related to the accumulation of acid products of anaerobic glycolysis produced during the hypoxic period. The center may become more sensitive to these products when the hypoxic period is terminated, more sensitive, that is, as the oxygen tension within the tissues is increased. Should the increased oxidations produce carbon dioxide in excess of that necessary to balance the buffer base freed by oxidation and conversion of the products of anaerobic glycolysis, the acidity of the center might well increase. Be that as it may, it appears that the postanoxic hyperpnea occurs while central $C^{(H^+)}$ may be decreasing (12). This change is generally considered as being adequate cause for a depression of respiratory activity. However, it appears likely that the increased oxygen made available to the respiratory neurones besides possibly effecting an increased carbon dioxide production serves to decrease the threshold of the center to (H^+) ions far more rapidly than the $C^{(H^+)}$ is decreased. If this situation actually exists it would explain the presence of hyperpnea where relative apnea might be expected. Furthermore, decreased oxidations accompanying the hypoxia might be expected to prevent respiratory adjustments to an increased $C^{(H^+)}$ should the sensitivity of the center be reduced by an anesthetic to a level which prevented the (H^+) ion stimulus from exceeding threshold strength at any time during the period of lowered oxygen tension. At this level of anesthesia, if severe damage has not been sustained by

the center during the period of oxygen lack, termination of the hypoxia should be attended by a stimulation of respiration. This actually occurred in two experiments, figure 1, sec. 2, B, and figure 2, sec. 1, E.

The long latent period of central hypoxic stimulation with its attendant depression of respiration also supports this thesis in that the respiratory depression occurs as the acid stimulus to breathing is increasing. It appears therefore that any increased respiratory activity that accompanies a given (H^+) ion increase during hypoxia could be considered as indicating a maximal response to the $C^{(H^+)}$ change by the center at a given level of oxidations and that the lower the oxidations, the smaller the respiratory response to a given increase in $C^{(H^+)}$.

The possibility that the stimulation of respiration attending hypoxia, in the absence of carotid and aortic and possible pulmonary chemoreceptor innervation, may be due to unknown chemoreceptive mechanisms cannot be ruled out by these experiments.

The occasional association of coördinated running movements, jactitation, and clonic convulsions (cyclopropane anesthesia) with the hyperpnea that attends hypoxia after complete chemoreceptor denervation suggests the excitation of motor centers besides the respiratory. These signs of generalized motor stimulation are seen under all states of innervation when the animals are very lightly anesthetized. When they do appear a carefully directed increase in the depth of anesthesia will entirely abolish them before the respiratory reactions are much reduced. In addition to this observation we have the fact that respiratory stimulation generally appears before the signs of general motor excitation (very occasionally they appear simultaneously) and considering further that some of the largest respiratory responses to hypoxia after denervation are entirely unattended by any sign of stimulation of other motor centers (fig. 1, sec. 2, C and fig. 2, sec. 1, F), it appears improbable that the stimulation of breathing is secondary to the stimulation of other motor centers. It seems reasonable to assume that the hyperpnea as well as the jactitation, running movements, and convulsions have a common stimulus, possibly an increasing cellular acidity.

However, it is not to be necessarily inferred from these experiments that the unanesthetized denervated animal will react in a similar manner, as there is a definite possibility, when the vagaries of all biological processes in anesthetic states are considered, that the central respiratory stimulation by oxygen lack may subsequently prove to be peculiar to the light anesthetic state.

An interesting observation is that the typical slow deep breathing following vagotomy is in most cases lost when the anesthesia becomes sufficiently light. This suggests the assumption of vagal function by respiratory muscle proprioceptive reflexes. One animal out of six, even when very lightly anesthetized, was unable to establish an eupneic type of respiration after vagotomy. All the others behaved as did the four described in this article. The observation that rapidly augmenting inspiration and a fast rate of respiration was the general finding in vagotomized animals when lightly anesthetized is in accord with the findings of Sharpey-Schafer (22) (23) and Tiitso (25).

SUMMARY AND CONCLUSIONS

Oxygen scarcity in the inspired air results in a significant sustained respiratory stimulation in lightly anesthetized animals[4] acutely deprived of known peripheral chemoreceptive and vagal proprioceptive reflex drive mechanisms.

The respiratory stimulation is generally preceded by a long latent period that is associated with depression of breathing and vasomotor pressor activity. The hyperpnea itself is characterised by periodicity, and a rapid rate. Readministration of room air is attended in most animals by a short period of respiratory depression. In all experiments, whether or not an immediate postanoxic depression occurs, a postanoxic hyperpnea obtains which differs from the hypoxic hyperpnea in that the attendant minute volume is greater. The postanoxic hyperpnea approximates in type the central stimulation of carbon dioxide far more than does the hypoxic response. The latter has the essential characteristics of anoxic stimulation in the innervated animal.

A very slight increase in the depth of anesthesia serves to reduce greatly or abolish the hypoxic response, but only diminishes the postanoxic hyperpnea. Further increase of anesthetic depth results in the often seen picture of hypoxia after complete chemoreceptive and vagal proprioceptive denervation, namely: progressive respiratory and circulatory depression that may be occasionally associated with a transient, inadequate increase in breathing.

The indirect evidence provided by these experiments appears to indicate that decreased oxidations within the center constitute a most important limiting factor of respiratory adjustments to changes in central $C^{(H^+)}$.

The occasional association of signs of generalized motor stimulation with those of respiratory center excitation is interpreted as indicating that functionally diversified motor neurones have a common sensitivity to increasing cellular acidity and not as indicating that the stimulation of breathing is secondary to increased activity of other motor centers.

Under sufficiently light sodium evipal and sodium pentothal anesthesia the rapidly augmenting inspiration of the normally innervated animal is present after vagotomy in the majority of animals. The slowly augmenting inspiration and slow rhythm described as being typical of breathing after vagotomy are always present when the anesthesia is sufficiently deep. It is suggested that respiratory muscle proprioceptives may take over the rôle played by the vagi if the anesthesia is light.

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THE INFLUENCE OF THE PITUITARY AND ADRENAL CORTEX ON RESISTANCE TO LOW ENVIRONMENTAL TEMPERATURES[1]

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It is becoming increasingly apparent that adrenal function is intimately concerned with various physiological processes which enable the organism to withstand stress. Detailed information in this regard may be obtained in those forms of stress that can be experimentally controlled, such as exposure to low environmental temperatures. Many workers have observed that adrenalectomized animals are unusually sensitive to cold. For example, in 1914 Elliot (1) found that adrenalectomized cats would survive for longer periods if kept in heated cages. Belding and Wyman (2) noticed the greatest mortality among adrenalectomized rats during periods when there was a fall in temperature in the animal house. Cannon and his school (3, 4) had already pointed out the intimate relationship of the adrenals to temperature regulation, while Wyman and tum Suden (5) later found that cortical transplants in adrenalectomized rats restored to normal their ability to maintain a normal rectal temperature when exposed for two hours in a moderately cold room, while rats with cortical insufficiency were unable to maintain their normal body temperatures. This demonstrated the relatively greater importance in this regard of the cortex over the medulla. Hartman, Brownell and Crosby (6) observed that the administration of cortin enabled adrenalectomized rats to maintain their body temperature almost as well as normal rats when placed in a cold environment and Selye and Schenker (7) have recently made use of this phenomenon in the development of an assay for small quantities of cortin. This sensitive test enabled Weil and Browne (8) to detect cortin in human urine.

Hypophysectomized animals are similar to animals deprived of the adrenals in exhibiting an impaired capacity to maintain a normal body temperature when exposed to cold as first observed by Smith and Foster (9, 10). Baird, Cloney and Albright (11) found that cortin would protect hypophysectomized animals under those circumstances, and concluded that the adrenals were largely responsible for the maintenance of body temperature during cold exposure.

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Although it is well recognized that the hypophysis is essential to the maintenance of the normal size and histological structure of the adrenal cortex, little is known concerning the action of hypophyseal corticotrophin on the adrenal cortical function. As the normal resistance to a cold environment requires an adequate functioning of the adrenal cortex, it appeared likely that this reaction would serve as a measure of corticotrophic activity in the hypophysectomized animal. In the present study the atrophic adrenal cortices of hypophysectomized rats were stimulated by the injection of pituitary extracts and the influence of this treatment was then tested under the stress of exposure to low environmental temperatures.

METHODS. The experiments reported here were performed on hypophysectomized rats whose ages ranged from 35 to 40 days. The weight of the animals varied between 70 and 90 grams. Male animals were used in all cases except in individual experiments when the factor of sex was being investigated. The hypophysectomy was performed by a parapharyngeal approach according to Greep's modification (12) of the technique of Smith (9); injections were usually begun immediately after operation and continued twice daily until the end of the experiment; body weights were recorded daily. After various periods of treatment and from 0 to 51 days after the operation, the animals were placed in a refrigerator at 0° ± 1°C. and at a relative humidity of 70 to 75 per cent. Each animal occupied an individual compartment in a cage of ½ inch mesh wire which was designed to allow free convection of air and unobstructed radiation to all sides except toward the limited area occupied by the other animals in nearby compartments. No forced circulation of air was supplied.

In some experiments adrenalectomy or thyroidectomy was performed and in other instances more than one endocrine organ was ablated. A total of 643 animals was used, 57 unoperated animals, 459 hypophysectomized, 72 adrenalectomized, 33 hypophysectomized-adrenalectomized and 22 hypophysectomized-thyroidectomized. The exposure to 0°C. was interrupted at hourly intervals for the measurement of colonic temperatures. These readings were taken in a room at 8°C. and required a period of 5 to 10 minutes, depending upon the number of animals under test. The duration of exposure to low temperature varied from 1 to 8 hours and was usually discontinued prior to the death of the animals. When survival was desired the animals were removed to their accustomed room temperature of 26 to 28°C. before their body temperatures had fallen below 15°C., otherwise subsequent fatalities were frequent. In individual experiments the animals were divided into two or more equal groups, so that untreated animals served as controls for each determination. This procedure was essential because tests upon similarly prepared animals on different occasions yielded dissimilar temperature curves no matter how carefully the above precautions were followed. This variation is shown in the figures below, but the source of the phenomenon has not been uncovered. One variable which could have been controlled more rigidly was the length of time required to read body temperatures. On the whole a longer period was required in the 8°C. room when larger groups were used and consequently these groups were less rigorously chilled than smaller groups.

At autopsy the adrenals, the thyroids, the gonads and the ventral prostates were weighed and the completeness of the hypophysectomies was checked by an examination of the sella turcica under a dissecting microscope. At the same time the base of the brain and the diaphragm of the sella turcica were carefully examined for signs of damage. Excluded from the data were animals incompletely hypophysectomized or animals in which the diaphragm had been ruptured during operation resulting in damage to the base of the brain. Such animals usually showed an inability to maintain their body temperature regardless of whether they had been treated or not.

In experiments where the significance of observed differences were questionable, Fisher's formula was applied and in the following discussion only those differences yielding a P value of 0.05 or less are stated to be significant.

Corticotrophic extracts were prepared from the whole pituitary glands of sheep. Freshly frozen tissue or commercial acetone-dried powders served as starting material. For the sake of satisfactory yields of the active material, acid or alkaline extraction media were employed at pH 1.5 to 3 or 8.0 to 10.0, respectively. The degree of purity and the freedom from other pituitary hormones varied widely in different preparations and no preparation used could be considered to be entirely pure. The preparation used in many of the experiments was made from fresh glands by the repeated extraction at pH 2 in 60 per cent acetone and subsequent precipitation of the active material with 4 volumes of acetone. This preparation was not further purified and is referred to below as crude acid acetone extract. Other extracts were prepared by the method of Lyons (13) but differing to the extent that no iso-electric precipitation of corticotrophin at pH 6.5 was performed. These extracts contained luteotrophin but were free of F.S.H., L.H. and thyrotropin and did not promote growth. Later experiments were made with more highly purified preparations virtually free of luteotrophin. In each case the dosages are expressed as weight equivalents of original acetone-dried whole pituitary powder. In this calculation it was assumed that the weight of the commercial dry powder was 20 per cent of the wet weight of the fresh gland. The corticotrophic activity of these extracts was determined by the adrenal weight increase in hypophysectomized animals injected twice daily for 5 days. In some cases these assay animals were tested in the cold immediately prior to autopsy.

Experimental. A number of groups of untreated male rats were exposed to cold under the above conditions at various intervals after hypophysectomy. The rates of fall in body temperature are plotted in figure 1, each curve representing the average temperature change of a group of 4 to 19 animals. In no case were these animals exposed to the cold more than once. It is apparent that determinations made on different occasions vary considerably even though like conditions as to age, size of the animals, temperature of the environment, and so forth, prevailed. The increased sensitivity to cold on the 4th and 28th day after operation is manifest, however, as is the striking resistance 24 hours after the operation. Animals exposed immediately after hypophysectomy were somewhat inferior to the 24 hour groups in their capacity to withstand the

exposure and were similar in their behavior to the groups tested 48 hours post-operatively. One group of adrenalectomized animals exposed on the day after operation is included for comparison. It would appear from these data that four days are required for the hypophysectomized animals to develop a degree of sensitivity to cold comparable to that of animals deprived of their adrenals. Female rats tested under these same conditions were found consistently to be more resistant to cold than males.

The influence of adrenal cortical extracts and of desoxycorticosterone acetate was tested on hypophysectomized and on adrenalectomized animals in the following manner. On the evening prior to the test and shortly after operation one injection of 1 cc. of cortin[3] or 0.5 mgm. of desoxycorticosterone acetate[3] in

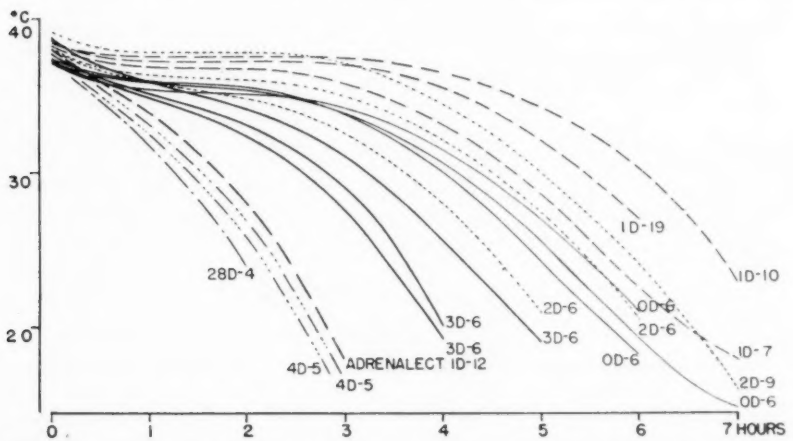

Fig. 1. Average rate of fall of the body temperatures of untreated hypophysectomized male rats exposed to an environmental temperature of 0°C. The numbers of animals used in each group is given. *0D* = on the day of hypophysectomy, *1D* = 1 day after hypophysectomy, and so forth. One group of adrenalectomized animals, exposed to cold one day after operation, is shown for comparison. The animals were most resistant to cold on the day following operation and reached their maximal sensitivity three days later. All animals were exposed to cold on one occasion only.

0.1 cc. of oil was given. A second like injection was made immediately before the animals were exposed to 0°C. Figure 2 shows the rate of temperature fall in 6 groups of animals. The cortin and desoxycorticosterone acetate treatment significantly increased the resistance of adrenalectomized animals. In the hypophysectomized animals cortin was definitely effective in this regard but in this experiment at least desoxycorticosterone was without effect.

In an extensive series of experiments hypophysectomized rats have been treated with pituitary extracts for periods of 1 hour to 14 days and their behavior in the cold environment compared with that of control untreated ani-

[3] The cortin and desoxycorticosterone acetate (doca) used in the experiments were kindly supplied by Roche-Organon, Inc., Nutley, N. J.

mals. The effect of the crude acid acetone extract in four experiments is shown in figure 3. A single injection of 25 mgm. equivalents of pituitary powder immediately after operation and 1 hour before exposure proved effective in exerting a statistically significant protection as shown in figure 3A. A similar response was noted in the animals shown in B which received two injections during the 24 hours between operation and exposure. A greater difference between control and injected animals was noted when treatment was continued for 2 and 3 days, respectively, in experiments C and D. It is of interest to note that the average adrenal weights shown in A and B of the treated and control animals are identical and that no significant increase in adrenal weight occurred until after 3 days of treatment at this dosage level.

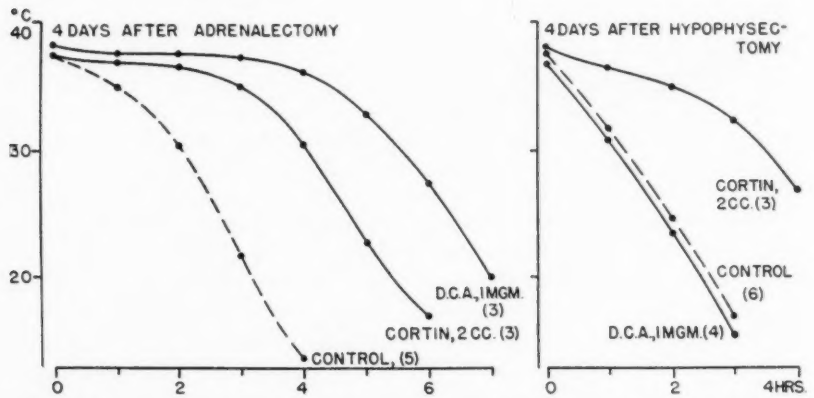

Fig. 2. The effect of cortin and desoxycorticosterone acetate on the rate of fall in body temperature of adrenalectomized and of hypophysectomized rats exposed to 0°C. During the 4 days between operation and exposure to cold the adrenalectomized animals were given 1 per cent NaCl in their drinking water. Two injections were made, 12 hours and immediately before the exposure to cold. The total doses are given in the figure. Protection was afforded both types of animals by cortin, but only the adrenalectomized animals were affected by desoxycorticosterone acetate.

When it was noted that considerable variation occurred between similarly prepared groups of animals it seemed advisable to introduce a further control measure. Prior to treatment hypophysectomized animals were exposed to cold and then on the basis of their performance in this preliminary test they were divided into two groups. One group was treated and the other saved as control. In 5 of 6 such experiments the less resistant group was injected with corticotrophic extract while the more resistant animals were allowed to go untreated. In this way a comparison could be made between injected and control groups as well as between the performances of the individual animals in the two tests. The data obtained in this way are given in figure 4. In experiments A, B and C the untreated animals were more sensitive to the cold on the second exposure while in D, E and F the untreated animals had already reached their maximal sensitivity when the first test was made 6, 21 and 28 days after operation. The

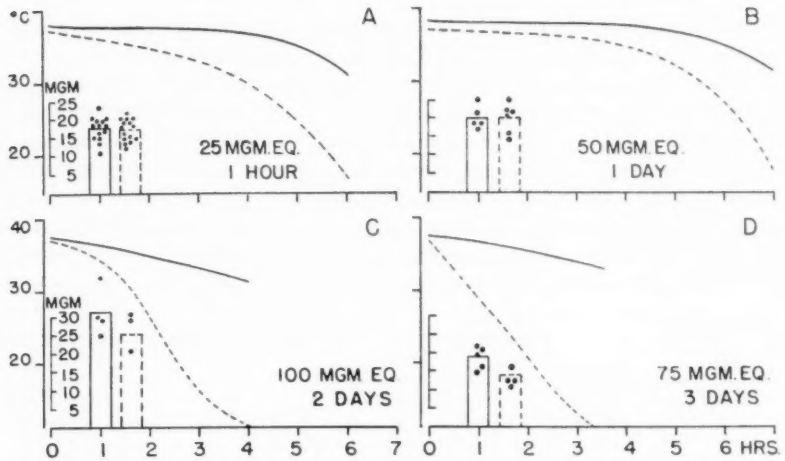

Fig. 3. Influence of crude pituitary extracts on the loss of body temperature of hypophysectomized rats exposed to 0°C. The treated animals (solid lines) were better able to maintain their temperature even when given a single injection one hour prior to exposure, but no adrenal weight change occurred until after 3 days of treatment. Young male animals of 70 to 90 grams were used in A, B and D; adult males of 270 grams in experiment C.

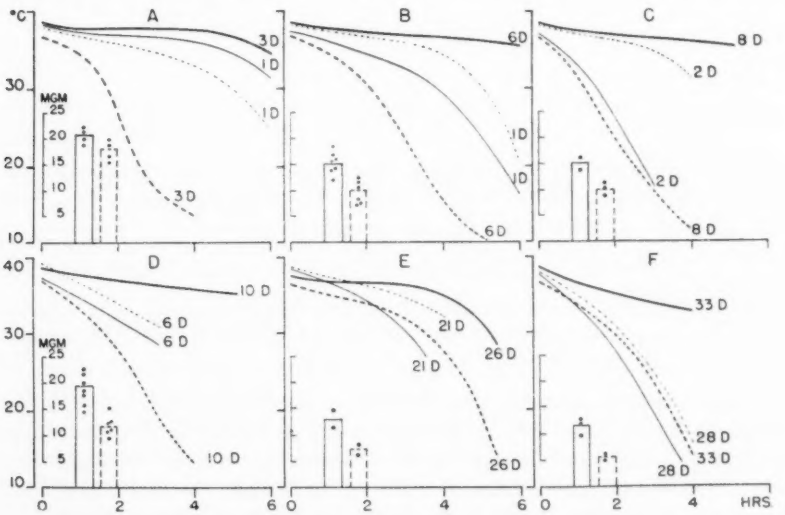

Fig. 4. Temperature curves of 6 groups of rats exposed to cold on two occasions. Between the first and the second exposure one half of the animals in each group were treated twice daily with corticotrophin. The performance of the treated animals is shown in heavy solid lines and of the untreated in heavy dashed lines. The behavior of these same animals on the preliminary test is shown in light solid and dashed lines, respectively. The number of days that elapsed after hypophysectomy is given in each case. The rectangles represent the mean adrenal weight in each group. Males were used in experiments A, B, F, and females in C, D and E.

injected animals showed improved temperature maintenance after treatment in each instance, irrespective of their performance in the preliminary test.

The experiments recorded above were performed with either the crude acid-acetone extracts or the extracts prepared by Lyons' method. Other confirmatory tests have subsequently been made using more purified preparations known to be free of F.S.H., L.H., thyrotropin, luteotrophin and the growth principle. However, assays have not been performed to exclude the presence in these extracts of the several postulated metabolic principles. Consequently further experiments were considered desirable in order to establish the site of action of the factor or factors concerned in this increased tolerance to cold. The first

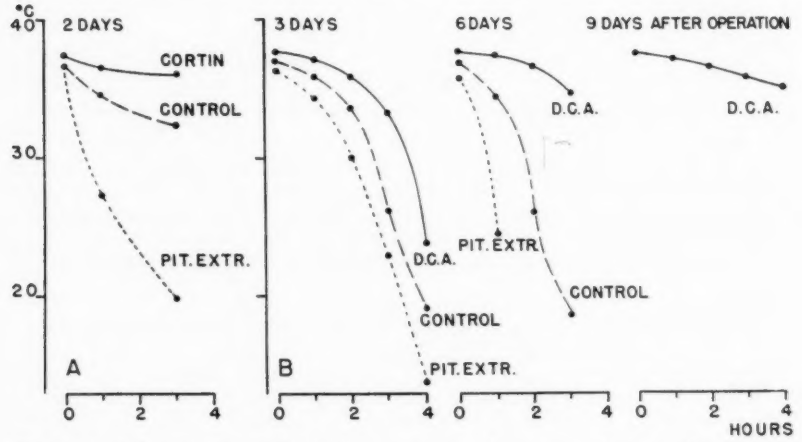

Fig. 5. A. Influence of a crude pituitary extract and cortin on the loss of body temperature of adrenalectomized rats, exposed to an environmental temperature of 8°C. two days after operation.

B. Effect of a crude pituitary extract and of desoxycorticosterone acetate on other groups of adrenalectomized rats exposed 3 times to 8°C., on the 3rd, 6th, and 9th successive day after operation.

Each group represents the average of 4 animals. Injections of 0.5 cc. of cortin, 0.25 mgm. of desoxycorticosterone acetate and of 25 mgm. equivalents of sheep pituitary powder, respectively, were made twice daily.

requirement was to determine the influence of pituitary preparations on adrenalectomized animals. Two such experiments performed at 8°C. are shown in figure 5. In A are shown three groups of animals. Cortin treatment in one group increased their resistance over the controls while the crude acid-acetone extract of proven efficacy in hypophysectomized animals, was deleterious. In figure 5B are shown three groups of animals exposed on three occasions. A similar response to that shown in A was observed after three days of treatment with desoxycorticosterone acetate. The difference became more marked after a further period of 3 days, and by the 9th day only the desoxycorticosterone-treated group still survived. Similar results were observed in repeated experiments with exposure of the animals to 0°C.

Although these data were strongly suggestive of an adrenal mediation of the observed action of pituitary extracts in hypophysectomized animals, they did not exclude the possibility that a part of the protection afforded hypophysectomized animals was centered elsewhere. It appeared highly probable that if the injected pituitary extracts exerted a protective action against cold in hypophysectomized animals by some other route than via the adrenal cortex such an action would be detectable in animals exposed to cold after both the adrenals and hypophysis had been removed. The data of figure 6, however, fail to show that any protection is afforded such doubly operated animals. Animals prepared in this way were highly sensitive to cold; they were protected by either cortin and desoxycorticosterone acetate but not by a crude pituitary extract or by a purified preparation of corticotrophin.

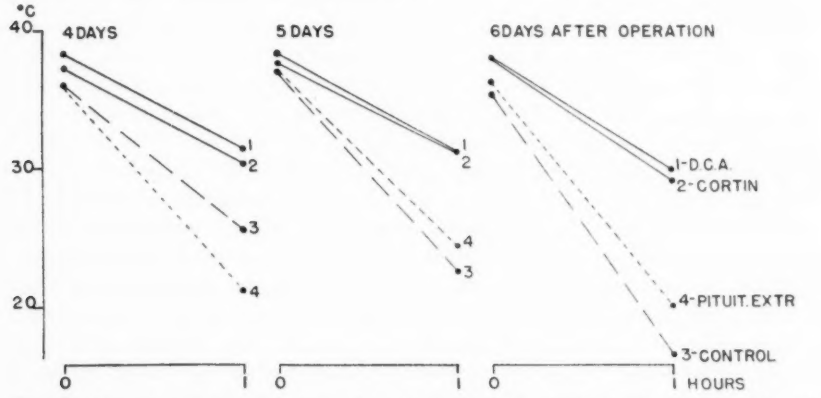

Fig. 6. Temperature curves of hypophysectomized-adrenalectomized rats injected with desoxycorticosterone acetate, cortin, and a crude pituitary extract, respectively, and exposed 3 times to 0°C., on the 4, 5 and 6th successive day after operation. Each group represents the average of 4 animals. Injections of 0.5 cc. of cortin, 0.25 mgm. of desoxycorticosterone acetate and of 25 mgm. equivalents of sheep pituitary powder, respectively, were made twice daily for the 6 days of the experiment. All animals were allowed a 1 per cent NaCl solution between tests.

A similar series of experiments was performed on animals thyroidectomized at the time of hypophysectomy. These two operations likewise resulted in a very low resistance to cold. However, in the absence of the thyroid the pituitary extracts were effective in increasing both the weight of the adrenals and the cold tolerance of the animals. Treatment of 9 animals twice daily for 6 to 10 days with 25 mgm. equivalent of acetone-dried sheep pituitary powder resulted in a 50 to 87.5 per cent increase in the adrenal weights over those of 10 controls. After exposure to 0°C. for two hours the average body temperature of the three injected groups was 30.7, 30 and 29.8°C., and that of the corresponding control groups 23, 23.9 and 19.5°C., respectively.

Discussion. The importance of a great many factors in temperature regulation is attested by the experience of numerous investigators. The present ex-

periments deal with the importance of the anterior pituitary and the adrenal cortex to the exclusion of other influences. Incidental to the experiments recorded above observations were made on certain other factors of importance. For example, animals younger than those used above exhibited a poikilothermic tendency which made them unsuitable for the tests. Large animals were better able to withstand cold than small animals, as were animals with a heavy growth of fur. Females were consistently better able to withstand cold than males. Lesions of the central nervous system accidentally inflicted during removal of the pituitary induced a sensitivity to cold which was more striking than that produced by any other single manipulation. These factors had to be excluded in order to obtain consistent results.

The experiments reported above indicate that the injection of pituitary extracts increases the resistance of hypophysectomized rats to cold. Purified extracts high in corticotrophic activity had an effect similar to cruder preparations and in either case no effect was observed in the absence of the adrenals. This is evidence for the belief that pituitary extracts exert their protective action through the adrenal cortex and that the enhanced adreno-cortical function is responsible for the increased tolerance to cold.

It is to be emphasized, however, that this treatment did not restore a normal response to low temperatures. The untreated hypophysectomized animals were unable to maintain their body temperatures above a lethal level for longer than 3 to 8 hours. Pituitary extracts permitted survival for perhaps twice this period but the capacity of treated hypophysectomized animals to withstand an environment of 0°C. falls far short of the 24 to 48 hours that can be tolerated by an unoperated untreated rat. Apparently, therefore, the removal of the hypophysis imposes a sensitivity to cold that is not completely corrected by the injection of pituitary extracts, at least not by extracts of the type and in the quantities used here.

The quite reasonable assumption that the pituitary may exert a protective influence against cold by some other route than over the adrenal cortex did not receive support from the experiments on adrenalectomized animals or on animals lacking both the hypophysis and the adrenals. Under such conditions pituitary extracts were without effect in increasing cold tolerance.

Many investigators have minimized the importance of the thyroid in increasing resistance against cold. The above data support this, for although thyroidectomy further increased the sensitivity of hypophysectomized animals, the absence of both glands did not prevent the protective action of pituitary extracts.

The experiments here reported confirm that immediately after hypophysectomy an increased sensitivity to cold is manifest. However, during the first 3 days postoperatively a certain resistance is retained and only after 4 days is the permanent high sensitivity to cold attained. This slow development of cold intolerance may be related to the similarly slow atrophy of the adrenal cortex described by Smith (9), and may indicate that some degree of adrenal cortical function continues even though anatomical regression is in progress.

It is of interest that a protective action against cold was noted immediately

after a single injection of corticotrophin. Such a result lends support to the current belief that in the normal animal the adrenal cortex responds promptly to stress by an increased activity and that this effect is brought about by an increased secretion of corticotrophin by the pituitary.

On the other hand the adrenal cortex retains its responsiveness to corticotrophin for many weeks after hypophysectomy as shown by the experiments in which both function and size was restored by pituitary extracts as long as 28 days after operation.

It has been shown (Ingle, 14; Selye, 15) that the hypertrophy of the adrenal cortex in the normal animal under conditions of stress may be considerable, amounting to a 25 per cent weight increase in the first 12 hours. The amount of corticotrophin given hypophysectomized animals in the present experiments was insufficient to bring about a comparable degree of hypertrophy, and it is reasonable to believe that herein lies the cause of failure to induce a normal resistance to low environmental temperatures.

SUMMARY

Hypophysectomized young rats weighing 70 to 90 grams were unable to maintain their body temperature when exposed to an environment of 0°C. Such animals became progressively more sensitive to cold over the first 4 postoperative days and thereafter exhibited a fairly constant response to cold. Crude pituitary extracts or purified corticotrophin increased the cold resistance of such animals, when administered for periods of from 1 hour to 14 days prior to exposure. These extracts were ineffective in the absence of the adrenals but were active after thyroidectomy. Adrenal cortical extracts increased the resistance of hypophysectomized rats and of similar animals without thyroids or adrenals. The protection against cold afforded hypophysectomized rats by pituitary extracts paralleled their corticotrophic activity and was attributable to the cortical function thereby induced.

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EXPERIMENTAL INVESTIGATION ON THE EFFECTS OF TRAUMA AND TRAUMATIC SHOCK ON GASTRO-INTESTINAL MOTILITY AND SECRETIONS[1]

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Little is known about gastro-intestinal motility and secretions during traumatic shock. Patients in this condition are known to vomit frequently, but this effect does not necessarily imply activity of the stomach. Moon has said that atony of the gastro-intestinal tract prevails during traumatic shock (1). In a recent study on the effects of burns we have found a great increase of gastric motility and, in a number of animals, a considerable increase in the volume and acidity of gastric secretion (2). We were, therefore, interested in investigating the effects of noxious agents other than burns, and report in this paper our findings of the results of mechanical trauma on gastro-intestinal functions.

EXPERIMENTAL PROCEDURES. Normal, healthy dogs were fasted for 30 hours, but had access to water. They were anesthetized with pentobarbital sodium or with ether and, following a suitable control period, trauma was administered to one or both hind legs by blows. Unless the animals died during the course of the experiment they were destroyed at its termination with an overdose of anesthetic. Blood pressure was recorded from the carotid artery with a mercury manometer, respiration by a cuff around the chest; intra-abdominal pressure by a balloon placed between liver and diaphragm, and gastro-intestinal motility by balloons placed in various organs. Manometers with a mixture of oil and carbon tetrachloride with a specific gravity of 1.5 were employed. For the collection of gastric secretion esophagus and pylorus were ligated, the latter just beyond the sphincter, and the contents of the stomach measured and titrated at the termination of the experiment. For the collection of other secretions the following ducts were cannulated: submaxillary duct, common duct with the cystic duct ligated, and main pancreatic duct. The secretions were recorded by an instrument described by us (3).

RESULTS. A. *Secretions.* a. *Salivary, Pancreatic and Biliary Secretions. Controls.* In 4 control experiments without trauma intravenous infusion of glucose (5 per cent)-saline (0.9 per cent) at the rate of 2 cc. per minute was administered by a constant injection pump previously described (4). Hourly intravenous injections of pilocarpine nitrate 0.25 to 0.5 mgm., or of secretin,[2] 5 to 10 mgm., were given alternately, and in the following "change of secretion"

[1] Aided by the Rosetta Josephson Fund.

Read as preliminary report at the 1941 meeting of the American Physiological Society.

[2] We are obliged to Dr. D. Klein, Wilson Laboratories, for a supply of secretin.

denotes changes in stimulated secretions. Salivary secretion did not change (3 expts. 12, 12 and 6 hrs.). Bile secretion did not change in one experiment (12 hrs.) and a slight decrease occurred in two (12 and 4 hrs.). Pancreatic secretion did not change in 3 experiments (12, 12 and 4 hrs.).

In all animals, controls and traumatized, blood pressure fell gradually as the experiments progressed, but the animals maintained a mean blood pressure of about 100 mm. Hg during the greater part of the experiment.

Trauma (19 expts.). Constant intravenous infusion was administered, as described above. In experiment 1, no drugs other than the anesthetic were used. Following trauma, biliary secretion dropped from 20 to 6 drops per 15 minutes,[3] pancreatic secretion from 1 to 0, salivary secretion from 1 to 0, and blood pressure from 140 to 90 mm. mercury. At the end of the experiment 0.5 mgm. of pilocarpine was injected intravenously and salivary secretion rose

TABLE 1

Gastric secretions following trauma

EXPERIMENT NUMBER	CONSTANT INFUSION PER MINUTE	BLOOD PRESSURE		GASTRIC SECRETION					REMARKS
		Before trauma	After trauma	Hours	Volume		Acidity		
					Total	Per Hour	Free	Total	
	cc.	*mm. Hg*	*mm. Hg*		*cc.*	*cc.*			
1	6			4½	15	3.3	0		Starved
2	6			4½	20	4.4	8	21	Starved
3	7			5¼	18	4	0		Starved
4	6	165/105	80/40 to 135/100	5	20	4	18	33	Starved
5	5	160	135 to 100†	5	Fecal material in stomach				Starved
6	7	165	75 to 125†	5	25	5	0	10	Fed
7*	6	115	40 to 100†	5	10	2	0	10	Fed
8	7	185/115	105/80 to 160/110	5	25	6	14	41	Fed
Averages				4.9	16.7	3.4			

* No. 7, ether; all others, nembutal anesthesia.
† Mean pressures.

to 54 drops, indicating that the potential function of the submaxillary gland had not been lost. A similar experiment was carried out on dog 2. Biliary secretion dropped from 15 to 9, pancreatic secretion from 1–3 to 0, salivary secretion from 20 to 0, and blood pressure from 120 to 40. In experiments 3–5, pilocarpine and secretin were administered as described under "controls." In experiments 3 and 4 pancreatic and salivary secretions were affected slightly or not at all by traumatic shock, while biliary secretion showed a 50 per cent decrease. In experiment 5, pancreatic secretion was not changed, while biliary secretion dropped 50 per cent.

b. *Gastric Secretion.* (Table 1.) Control experiments without trauma were performed on ten animals, in order to test the effects of anesthesia and operation. The rate of gastric secretion varied between 2.1 and 3.4 cc. per hour, with an average of 3.0 cc. No free acid was present in 9 experiments, and a trace of

[3] In the following the figures for secretion represent 15 minute periods.

free acid in one. Total acidity varied between 10 and 20 degrees (one degree represents cubic centimeters of n/10 HCl per 100 cc. of gastric juice). The constant intravenous infusion of saline-glucose solution or feeding 10 hours before the experiment did not affect volume or acidity of gastric secretion, the average rate of secretion being 3.4 cc. in 7 animals.

Eight dogs under nembutal or ether anesthesia received constant intravenous infusion of saline (0.9 per cent) glucose (5 per cent). Four to five hours following the trauma the stomach was excised and its contents collected. The hourly rate of secretion varied between 2 and 6 cc. A small amount of free acid, varying between 8 and 18 degrees was found in 3 experiments; total acidity varied between 10 and 41 degrees. Previous starvation for 30 hours, or feeding 12 hours before the experiment, did not seem to affect acid secretion following trauma. A comparison with controls without trauma shows that the volume of secretion was not affected by trauma, the average hourly rate of secretion being 3.4 cc. in both series. We do not feel that the small amount of free acid found in 3 of the experiments with trauma was significant.

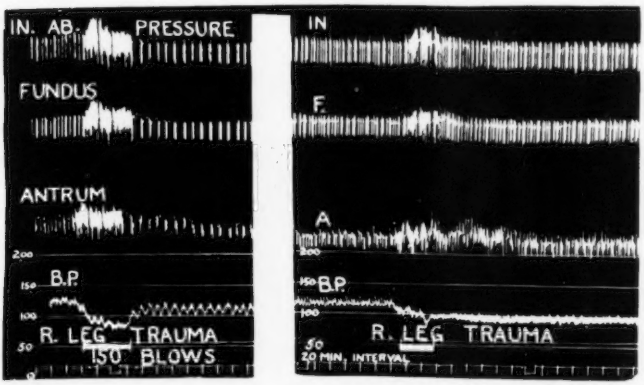
c. *Motility.* (Figs. 1 and 2.) Five dogs under nembutal and one under ether anesthesia were used. Blood pressure was recorded from the carotid artery, and balloon records of the motility of the distal part of the stomach (antrum) and, in some dogs, also of the body of the stomach, of the duodenum and of the colon were obtained. In one animal the motility of the exposed stomach was observed directly. After a suitable control period trauma was administered to the legs.

In 3 experiments (nembutal) antrum motility and tone were increased for short periods following trauma; in one of these experiments, in which colonic and duodenal motility was registered also, an increase of these motilities was noted. In 2 experiments (one nembutal, one ether), antral and duodenal tone and motility were diminished following trauma. In one of these experiments a slight and short increase of antral motility appeared after the sixth and seventh trauma. Following another traumatization, depression of antral motility occurred.

Figures 1 and 2 represent two typical experiments. In the experiment of figure 1, antral motility became greater after additional traumatizations, and when blood pressure was rather low, i.e., at 50 mm. Hg and below; at this stage, manipulation of the traumatized limbs was followed by prolonged antral motility.

In experiments in which antral motility was observed to follow trauma, the degree and duration of this motility was rather small as long as blood pressures were above 80 mm. As the experiment progressed, and further trauma or manipulation of the traumatized areas reduced blood pressures to 50 and below, motility of the antrum became stronger and more prolonged. The effects of trauma on antral motility were inconstant, however. This is demonstrated in figure 2.

In the experiment in which the stomach was observed directly, a slight increase of tone and motility appeared 5 minutes after traumatization. Most



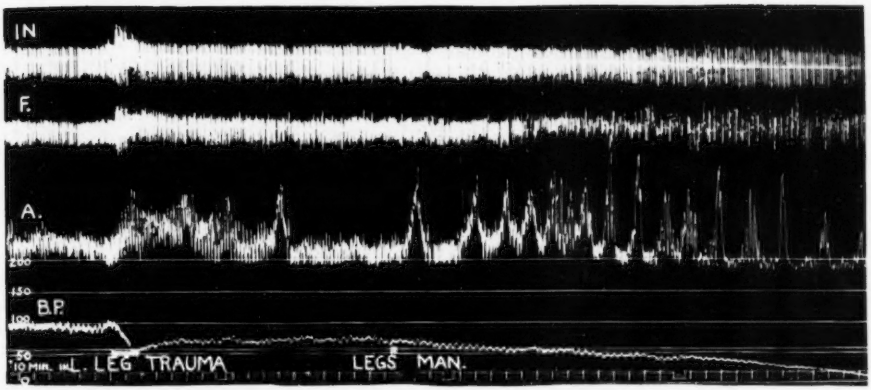


Fig. 1. Effect of trauma on gastric motility. Distinct increase of antral tone and motility following trauma to left leg and manipulation of traumatized legs; note low blood pressure. Nembutal anesthesia.

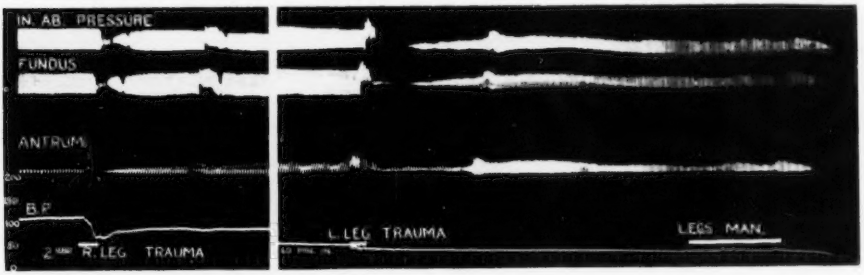


Fig. 2. Effect of trauma on gastric motility. Distinct depression of motility of the antrum following trauma of right and left leg. Ether anesthesia.

waves of contraction were seen in the body of the stomach, and fewer in the antrum.

Intra-abdominal pressure and respiration were increased slightly and for short periods of time following the trauma in some animals, while in others it was depressed.

Following trauma, slight edema and some petechial hemorrhages were found in the mucosa of the stomach and duodenum of two animals. A varying degree of anemia of the stomach and intestines was found, apparently depending on the severity of trauma and shock. The adrenals did not show gross changes.

Discussion. In the experiments in which secretion had not been stimulated, salivary, biliary and pancreatic secretions decreased, while in the experiments in which secretion had been stimulated, salivary and pancreatic flow were not changed, but biliary secretion diminished 50 per cent. Secretin was not a particularly strong stimulant for biliary secretion, however.

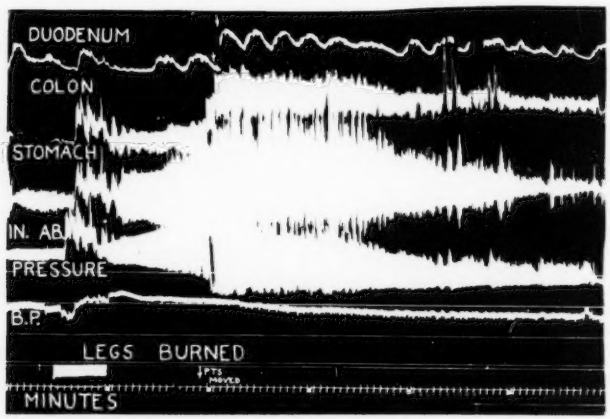


Fig. 3. Intra-abdominal pressure following burn. Nembutal anesthesia

We feel that changes in stimulated secretions may give more information about the function of an organ, because the periodic activities of most glands, as well as the effects of local changes in blood flow, may obscure functional changes. The depressive effect of burns on salivary, pancreatic and biliary secretions was considerably greater than that of mechanical trauma (2).

Gastric secretion was not affected significantly, neither in volume, nor in acidity, by various degrees of traumatization and ensuing shock. Gastric motility was slightly increased for short periods of time when the blood pressure of the traumatized animal was above 80 mm., and for somewhat longer periods and to a greater extent, when the blood pressure declined further. The intensity of gastric and mainly of antrum motility following trauma did not compare with that following burns. In the latter case it was seen mainly in the antrum, it was very forceful, began immediately with the burning and usually continued for prolonged periods of time. In no burn experiment was depression

of gastric motility observed, as seen in a number of experiments with trauma, but strong gastric motility was seen to follow every burn.

There is a considerable difference also between respiration following trauma or burns: with trauma, respirations may be inhibited or augmented, and in the latter case, the increase is of short duration and never as marked as with burns. Following burns, respiration is forceful and of great depth, and intra-abdominal pressure is greatly augmented (2); the latter never was seen to follow trauma in any of the experiments reported in this paper, nor in 50 similar experiments not reported here. The increased tone and contractions of the thoracic and abdominal musculature which were observed in nearly all burn experiments, never occurred with trauma. For comparison, the considerable increase of intra-abdominal pressure and respirations following a burn is demonstrated in figure 3 (unpublished experiment. Nembutal anesthesia).

There are thus fundamental differences between the manifestations of shock following trauma and burns, i.e., in respiration, intra-abdominal pressure, gastro-intestinal motility and gastric, pancreatic and salivary secretion. This demonstrates that although changes in circulation and composition of the blood may be similar in every case of fully developed shock, whatever its cause, other manifestations may be different. We believe that gastric motility following traumatic shock may be related to anoxemia, as it became more apparent with lower blood pressures. Of course, anoxemia of the stomach may be more dependent on vasomotor regulation than on systemic blood pressure, but experimentation only will be able to answer this.

SUMMARY

The effects of mechanical trauma on gastro-intestinal functions were studied in anesthetized dogs: stimulated salivary and pancreatic secretions were not affected much, but biliary secretion decreased. Trauma did not affect gastric secretion, and its effects on gastric motility were slight and variable. The effects on respiration and intra-abdominal pressure were variable and of short duration.

The effects of mechanical and of thermal trauma on gastro-intestinal functions are very different, and it is pointed out that in shock from different causes, certain manifestations may be quite different.

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THE EFFECT OF DENERVATION ON THE FILTRATION RATE AND BLOOD FLOW IN DOG KIDNEYS RENDERED HYPEREMIC BY ADMINISTRATION OF PYROGEN

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The discovery that certain pyrogens, such as those found in typhoid vaccine (3) or in contaminated inulin (9), can cause a marked increase in renal blood flow in man, even when fever is prevented by an antipyretic, raises the question of the mode of action of these pyrogens. [illegible]hey humoral agents or is their action mediated through the nervous system? Since they do not have any immediate action on blood pressure or renal blood flow after intravenous injections (9), they do not fall in the category of common depressor or vasodilator agents. The investigations described below were designed to explore this question. They also contribute to the growing body of evidence indicating that the circulation of the kidney under normal conditions is not under tonic nervous control (9).

METHODS. The ureters of two dogs were explanted to the exterior by the method of Danilov as described by Grabfield (8), making possible the collection of urine from each kidney separately. Creatinine clearance, as a measure of the glomerular filtration rate, and diodrast clearance, as a measure of effective renal plasma flow (6), were determined under [illegible]rmal conditions and during the renal hyperemia induced by intravenous adm[illegible]istration of 100 mgm. of the same lot of pyrogenic inulin (Pfanstiehl 268) [illegible]ed by Smith and his collaborators (9). In all these experiments the febrile reaction was blocked by a course of amidopyrine (four 5 grain tablets during the 12 hr. period before the experiment).

During an experiment the dog stood on a table supported in a sling. Diodrast and creatinine were administered by slow (0.8 cc. min.) intravenous infusion, and urine was collected by funnels placed under the ureter openings. Every effort was made to avoid obstruction of the ureters by pressure on the abdomen and all clearance periods in which the creatinine clearance showed marked irregularities which might have been due to obstruction have been discarded.

Creatinine concentration in cadmium filtrates (5) of plasma and in diluted urine was determined by the method of Folin (4). Diodrast concentration in cadmium filtrates of plasma and diluted urine was determined by the method of Alpert (1).

After a series of normal and hyperemic experiments the left kidney of each dog was virtually denervated by removing a section of the left sympathetic trunk. In dog 1 the trunk was removed from just posterior to the stellate ganglion in

the thorax to the second lumbar ganglion. In dog 2 the trunk was removed from just below the diaphragm to the second lumbar ganglion and in addition two splanchnic branches were located and interrupted by excising an inch or so. In both cases the interruption of the sympathetic trunk was verified at autopsy.

After the operation another series of control and hyperemic studies was carried out within six weeks to minimize the effect of regeneration.

RESULTS. There were no physiological effects of denervation on renal function. The denervated kidneys behaved, in respect to urine flow, filtration rate, and blood flow, like the normal kidneys. In figure 1 the creatinine/diodrast clearance ratio (taken to be equal to the percentage of plasma filtered at the glomeruli) is plotted against the diodrast clearance ex-

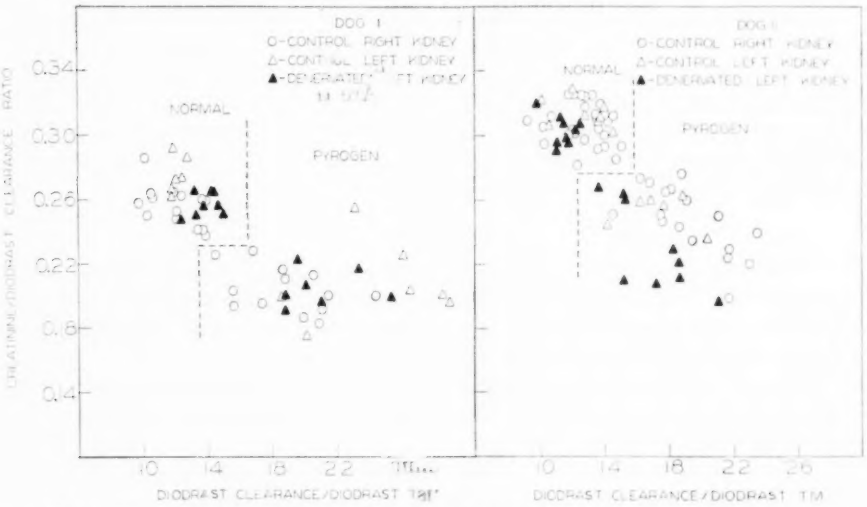

Fig. 1. The filtration fraction (creatinine/diodrast clearance ratio) in relation to the effective renal plasma flow (diodrast clearance per unit of tubular excretory mass (diodrast Tm) in the intact and denervated kidneys of two dogs under normal conditions and after intravenous pyrogen. Each datum represents a clearance period.

pressed per unit of diodrast Tm. This latter is taken to be a measure of the effective renal plasma flow per unit of functional tubular tissue (6).

The correspondence of the denervated kidneys with the normal control values is close, both at normal and at hyperemic levels of renal blood flow. The denervated kidney of dog 2 showed a tendency toward a decreased blood flow which may be correlated with a partial obstruction of the ureter found at autopsy. Although the ureter and pelvis were expanded, histological examination of this kidney showed no signs of pathological change due to this partial obstruction.

In each dog one control experiment has been omitted from the chart because it was divergent from the other control experiments; in one case there was an anomalous hyperemia, in the other an unusually high filtration fraction.

DISCUSSION. The dog kidney responds like that of man and the seal (2) to inulin pyrogen, showing an increased renal blood flow and a decreased filtration fraction. Smith *et al.* (10) have postulated that this combination of events indicates dilatation of the efferent glomerular arterioles.

The fact that the denervated kidneys respond like the normal kidneys to inulin pyrogen indicates that the ultimate action of this agent is humoral in nature, but whether it exerts a direct effect by delayed action on the renal arterioles, or acts indirectly causing the secretion of some physiological vasodilating agent, cannot be answered by these experiments.

The failure of the denervated kidney to differ from the nondenervated control kidney in the same animal confirms the evidence cited by Smith in his review of this subject (10) that the renal nerves play no significant part in controlling the renal blood flow (or the urine flow) under basal conditions. As pointed out there, the contrary view was based chiefly on acute experiments in which the sympathetic nervous system had presumably been excited to some activity by anesthesia and traumatic procedures. Our observations are in agreement with those of Goldring *et al.* (7) on a single case of a human subject with unilateral sympathectomy, not only with respect to the effect of denervation but also the response to pyrogen.

SUMMARY

1. The filtration rate (as measured by the creatinine clearance) and the renal plasma flow, (as measured by the diodrast clearance) of the separate kidneys of two dogs with explanted ureters was determined under normal conditions and during the renal hyperemia caused by pyrogenic inulin.

2. Later one kidney of each dog was denervated by removal of a part of the sympathetic trunk on one side. The clearance experiments were repeated. No effect of denervation was observed.

3. It is concluded that the action of pyrogenic inulin in causing renal hyperemia does not involve the sympathetic nerves to the kidney.

4. It is demonstrated that in the quiet, unanesthetized, standing dog, there is no tonic sympathetic control of the renal vascular bed.

5. Denervation does not affect the urine flow.

We are indebted to Prof. H. W. Smith for suggesting the problem and for his valuable advice, to Dr. Philip Grabfield for explanting the ureters, to Dr. John H. Mullholland for performing the denervations and to Dr. Irving Graef for examining the animals at autopsy.

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WATER INTOXICATION OF THE FROG (RANA PIPIENS)[1]

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Former studies (5, 7) of water intoxication have been confined to mammals: cats, dogs, guinea pigs, rabbits, rats and man. An excessive amount of water introduced orally, rectally, subcutaneously, intravenously or intraperitoneally, regularly produces symptoms of intoxication, among which are asthenia, restlessness, frequent urination, diarrhea, nausea, retching, vomiting, frothing at the mouth, stupor, coma and convulsive seizure resulting in death. When a volume of water equivalent to one-third of body weight has been given, mild symptoms appear; and with two-thirds of body weight, pronounced toxic symptoms develop. Recovery follows if the administration of water is discontinued or if a hypertonic saline solution or an extract of the adrenal cortex is injected.

During this intoxication of mammals the water content of all organs shows a marked increase, that of the liver being the greatest. The plasma volume increases while the electrolytes of the blood, potassium, calcium, sodium, and chlorides decrease. The exits of sodium chloride are in the vomitus and urine. All organs except the liver show a decrease in sodium chloride, that of the muscles is largest. The conclusion is that an ionic disturbance in the cells of the body is the basis of the mechanism of water intoxication.

In view of the remarkable toxicity of excessive amounts of water in mammals it seemed to us that the frog, due to the fact that it spends a large proportion of its life in water, might have developed a more adaptive water balance regulation and might yield different results when subjected internally to excessive amounts of water.

Males of the common green frog, *Rana pipiens*, in the winter condition were used throughout this investigation. During the first series of experiments the water was administered through a stomach tube. Usually a large portion of each administration was vomited immediately. That much was retained was evidenced by the results. Each animal received from 60 to 90 administrations of from 2 to 4 cc. of tap water more or less evenly distributed over 3 or 4 days. In spite of the fact that the frogs were given a relatively larger volume of water and over a longer period of time than were mammals, yet they survived.

A number of the animals were killed for examination. Gross inspection

[1] This investigation was supported by a grant from the Denison Research Fund of Wesleyan University.

The authors are indebted to Prof. Ross A. Gortner, Jr. for helpful advice on chemical procedures.

showed the stomach and intestine distended with water and gas to approximately 2 to 4 times normal size; the liver was slightly enlarged, flaccid, and lighter in color; the kidney, enlarged and flaccid, and the body cavity filled with an abnormal amount of fluid.

Histological studies showed the liver sinusoids dilated, particularly in the vicinity of large central veins. The sinusoids were filled with erythrocyte "ghosts." Individual and groups of hepatic cells were swollen and often cloudy. There was no evidence of nuclear or cytoplasmic destruction.

In the kidneys Bowman's capsules were enlarged and not completely filled by the gomerulus. Tubules were somewhat distended, while the intertubular spaces contained erythrocyte "ghosts." The alimentary canal showed the effects of dilatation. The vessels of the submucosa contained abnormal amounts of blood. The blood erythrocytes were somewhat swollen without indication of hemolysis. No other organs and tissues showed appreciable change.

Subcutaneous administration. When it became evident that oral administration was ineffective in producing evidence of severe intoxication, subcutaneous injections were used. The periodic injection of 2 cc. of water into the large lymph sacs resulted in complete retention of the water. With this method water was given at the rate of 5 to 6 per cent of body weight at intervals of from 30 to 60 minutes until the total ranged from 230 to 400 per cent of the body weight. Workers with mammals usually found death to occur before as much as 100 per cent of the body weight had been administered. Even with the extraordinary amount injected subcutaneously none of the intact frogs died of water intoxication.

At no time were nausea, gagging or vomiting observed during this method of administration. As the injections proceeded the animals gave evidence of depression in a change of body posture and in curtailed activity. There were no convulsions but eventually many sank into a stupor.

Morphological and histological examinations revealed, except in the alimentary canal, which was not affected, conditions similar to those obtained with oral administration. The damage to the liver and kidney was more severe but similar to that of the former method. The liver cells appeared to have lost large portions of cytoplasm, while the nuclei and cell membrane remained intact. The Bowman capsules of the kidney were enlarged by from 2 to 3 times the normal size, often with a separation of the two layers of cells. A heavily staining material suggestive of hemolyzed blood often filled the capsule.

Hypophysectomized frogs. Since it is well known that the posterior lobe of the hypophysis plays a rôle in the control of elimination of water, it seemed reasonable to assume that water intoxication might prove to be more harmful to the hypophysectomized than to the normal animal. After some practice the removal of the gland was easily accomplished. Our criterion for the completeness of hypophysectomy was that established by Hogben and Winton (2). They found that complete removal resulted in a pale condition because of a maximal contraction of the melanophores due to the loss of hormone, while on the other hand partial removal rendered the animal decidedly dark.

After operation we allowed a period of from 7 to 10 days for recovery and then induced water intoxication in the frogs by hypodermic injection. The resulting symptoms were severe and somewhat similar to those reported as characteristic in mammals. For the first 20 to 30 cc. of water injected, no appreciable variation from the condition of the intact control frog was observed. However, when from 40 to 80 cc. of water had been administered, muscular tremor and twitching were noted. These were accentuated by handling. Later typical convulsions occurred. If then the injections of water were continued the animal became powerless and death ensued. On the other hand, when administrations were stopped at the onset of convulsions a slow recovery occurred that required as much as a week to 10 days.

Chemical studies. These included the determination of organ and body water, urine and organ chloride, and urine and blood calcium.

The following methods were employed. For water content the organs, after the animal was bled, were removed, laid in filter paper and rolled around to absorb adherent water, then dropped into weighing bottles and dried at 105 to 110°C. for from 36 to 48 hours.

Urine chlorides were determined by the Volhard-Arnold (10) titration method; body chlorides by a method worked out by R. A. Gortner, Jr., and tissue chlorides by the method of Van Slyke and Sendroy (9).

Sodium of the urine was observed by the micro-titration method of Kramer and Gittleman (3).

The urine calcium was estimated by the micro-titration method of Tisdall and Kramer (6) and the blood and urine calcium by the manometric determination of Van Slyke and Sendroy (8).

Water balance. The animal under observation was kept in a specially constructed urine chamber that was placed in a large moist chamber. This made it possible to follow the changes in body weight. When the frog was to be weighed and injected with water, it was first picked up and held over the funnel of the urine chamber while its own random movements caused the passage of urine. It next was weighed and then injected with distilled water. When injections were continued throughout a period of from 3 to 4 days, they were omitted during the night for from 8 to 10 hours. During the day the body weight rose steadily with the first 6 or 8 injections by between 10 and 25 per cent. With the first 3 or 4 injections practially no urine was passed. After this the elimination steadily rose until it approximately equalled the amount of water per injection. In the mornings, following the overnight opportunity for recovery, the body weight was found to be somewhat greater than the initial weight. Furthermore, the morning recovery weights were always slightly above those of the preceding day. Thus was indicated the establishment of a water balance at succeeding higher levels. This may be construed as an adaptation on the part of the frog to the excessive volumes of water. This fact was observed in all cases in which the experiment lasted 3 or more days.

When hypophysectomized frogs were used, the water balance was carefully investigated in view of the diabetes insipidus that has been produced experi-

mentally in mammals by hypophyseal operations. When the increase in body weight of the completely hypophysectomized frogs and that of the controls were plotted the curves were similar, which would not have been the case if the hypophysectomized animals had developed diabetes insipidus. No evidence of the polyuria of diabetes insipidus was present in any of our operated animals. Hence it is concluded that the problem of water balance was met in a similar manner by the two experimental groups.

That the water content of the two groups was practically the same is indicated by the water content of the brain, kidney, and liver (see table 1). These results, with the exception of those for the brain, are in accord with mammalian observations reported by Rowntree and others. Rowntree (5) reported that in a water intoxicated dog the intracranial pressure was increased by an amount equivalent to 35 mm. of water. He observed marked edema in the liver, portal veins, brain, and renal cortex.

It is concluded that, although the hypophysectomized frogs were more severely damaged by water injections than were intact animals, the water content of the two groups was similar. The curves of change in body weight and the output of urine of each group were surprisingly similar. An outstanding difference between the reaction of mammals when compared with the frog is that the latter maintains its normal content of water in the brain, while the former experiences edema of that organ.

TABLE 1

Water content, in percentage

CONDITION OF FROG	BODY	BLOOD	BRAIN	KIDNEY	LIVER
Normal	79.1		83.2	82.2	70.8
Intact, H_2O intoxicated	81.2	87.1	83.5	85.0	75.8
Hypophysectomized, H_2O intoxicated		87.9	83.6	84.5	76.5

Urine chlorides. All determinations were made for 10 cc. of urine. The normal samples were pooled urine from many frogs fresh from the tanks filled with running tap water which contained chlorides. The chloride content of urine samples taken at different times ranged from 0.5 to 0.9 mgm., average 0.73 mgm. These values were regarded as normal in that the conditions for intake were adequate. Samples of the urine of water intoxicated animals were secured as each animal remained in the moist atmosphere of the urine chamber, in which there was no opportunity for NaCl absorption. The samples were taken at irregular intervals as it was necessary to wait until an animal had urinated from 10 to 13 cc. The output per sample ranged from 0.5 to 7.4 mgm. (table 2). It seemed that during the progress of water intoxication the control of body chloride was at first broken down but that later there was an attempt to conserve a minimum compatible with life (see frog 27, table 2).

In order to test the above conclusion the entire carcasses of normal and water intoxicated frogs were analyzed for chloride. The results are given in table 3.

The intoxicated frogs after having received approximately 400 per cent of body weight of water by subcutaneous injection were allowed to dehydrate for 10 hours; then were killed, desiccated at 104 to 108°C., and dissolved in concentrated HNO_3. The chloride loss by the intoxicated frogs, W38 and W39, was 33.5 and 46.5 per cent respectively of the average content of normal animals. Hence it is evident that a considerable amount of chloride remained in the body of severely intoxicated frogs.

The chlorides of the urine of five hypophysectomized frogs were determined during the period of water intoxication. The content of 10 cc. samples of urine gave results that corresponded to those obtained with intact intoxicated animals. There was in three cases a distinct rise and fall in the chloride concentration

TABLE 2

Chloride concentration in 10 cc. of urine

	I	II	III	IV
	mgm.	*mgm.*	*mgm.*	*mgm.*
W. 23	1.3	3.2	6.4	3.8
W. 27	1.1	7.4	1.5	0.7
W. 32	1.6	2.3	1.4	
W. 38	2.2	0.9	1.8	1.8
W. 39	1.9	2.0	0.7	0.5

TABLE 3

Chloride content of normal and water intoxicated frogs

ANIMAL	BODY WEIGHT	DRIED WEIGHT	TOTAL CHLORIDES	DIFFERENCE FROM NORMAL AVERAGE	LOSS OF BODY CHLORIDE
	grams	*grams*	*mgm.*	*mgm.*	*per cent*
Normal 1	31.8	6.5	28.7		
Normal 2	31.0	6.6	27.5		
W.I. 38	34.7	6.5	18.7	9.4	33.5
W.I. 39	33.7	6.5	15.0	13.1	46.6

similar to frogs W.27 and W.39 in table 2. Hence it may be concluded that the loss of chlorides was similar in the two groups studied.

Organ chlorides. Misawa (4) subjected rabbits to water intoxication and studied the chloride losses in the various organs of the body. All organs, save the liver, showed a decrease. This ranged from 20 to 51 per cent. We determined the chloride content of the liver, kidney, and brain of frogs subjected to as much as 400 per cent of water. Each organ lost chlorides; the liver 31, the kidney 15.5, and the brain 10 per cent (see table 4). These results suggest that the frog does not release chlorides as readily as rabbits. In table 4 light and heavy intoxication are compared. It should be noted that the liver lost chloride under both treatments.

Calcium. Since the excretion of water and chlorides was not greater among the hypophysectomized than among intact frogs, it occurred to us that the

calcium ion might be involved in the production of convulsions. Charles (1) found that in an African toad, *Xenopus laevis*, the removal of either the anterior lobe alone or of both lobes of the pituitary body caused a persistent fall in serum calcium.

Tests for calcium in the urine of water intoxicated intact and hypophysectomized frogs were negative. Hence blood examinations were made of pooled blood of two or three animals. Removal of the pituitary gland of itself was found to cause a slight fall in blood calcium (table 5). Water intoxication reduced the blood calcium of intact animals from an average of 7.7 to 6.9 mgm. per 100 cc. of blood. In water treated hypophysectomized frogs the average

TABLE 4

Organ content of chlorides of normal and water intoxicated frogs, in milligrams per gram of organ

CONDITION OF ANIMALS	BRAIN	KIDNEY	LIVER	MUSCLE
Normal	7.78	8.61	4.04	2.23
Light W.I.	7.65	7.70	3.72	
Heavy W.I.	6.98	7.26	2.79	1.96

TABLE 5

*Calcium content of whole blood of frogs in milligrams per 100 cc.**

INTACT		HYPOPHYSECTOMIZED	
Normal	Water intoxicated	Normal	Water intoxicated
7.2	6.8	5.9	5.7
7.4	6.1	7.4	6.6
8.1	8.0	6.5	6.6
7.4	6.2	7.0	5.5
8.2	7.2		5.7
			5.3
Average....7.7	6.9	6.7	5.9

* Each determination required the blood of 2 or 3 frogs.

was 5.9 mgm. In 12 of 18 animals it was 5.7 mgm. or less. Waggener (11) observed that after removal of the parathyroids in frogs a drop occurs in the blood calcium which is equivalent to about $\frac{1}{4}$ to $\frac{1}{3}$ of the total calcium normally present. In our water intoxicated hypophysectomized animals the drop ranged from 15.4 to 32.1 per cent. The drop in calcium may, therefore, explain the developemnt of convulsions among water intoxicated hypophysectomized frogs.

SUMMARY AND CONCLUSIONS

Frogs in the winter condition withstand water intoxication better than mammals. Water administered to the normal animal by stomach tube or subcutaneously at intervals of 30 to 60 minutes in doses of 2 to 4 cc. over a period of 3 days failed to kill them.

Histological studies revealed severe damage in the kidney and liver, but this was not permanent. Complete recovery occurred within 10 to 14 days.

Hypophysectomy renders frogs susceptible to water intoxication. When they have received from 40 to 80 cc. of water, muscular tremor and twitching appear. If injections are then continued convulsions occur and they die.

The water content of the body and organs of the intact and hypophysectomized water intoxicated frogs rose approximately equally. The brain did not show hydration.

The excretion of chlorides during water administration at first rose but later decreased. It is suggested that this is an attempt by the body to conserve a minimum compatible with life.

The calcium content of the blood in water intoxicated frogs was lower in the hypophysectomized than in the intact animals. In the water intoxicated hypophysectomized frogs the drop in blood calcium often was as large as a third of the normal content. Since others have reported that a drop of from $\frac{1}{4}$ to $\frac{1}{3}$ of the total blood calcium causes tetanus in parathyroidectomized frogs, it is suggested that the similar drop in water intoxicated hypophysectomized frogs also causes convulsions and death.

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THE RESPIRATION OF NEURONES[1]

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A considerable literature now exists dealing with the gross respiration of the central nervous system, and evidence is accumulating on the respiration of structurally delimited regions (Gerard, 1938). Experiments of the latter type are mostly indirect, metabolic intensity being inferred from vascularity (Wolff, 1938; Craigie, 1941; Scharrer, 1939), resistance to anoxia (Sugar and Gerard, 1938; Kabat, Dennis and Baker, 1941; Fazekas, Alexander and Himwich, 1941; van Harreveld, 1941) or hypoglycemia (Tyler, 1940; Gellhorn, Packer and Feldman, 1940), concentration of oxidizing enzymes (Campbell, 1939), rates of reaction with special oxidation systems (ferric chloride, Gerard, 1938; ferricyanide, Michaelis and Quastel, 1941), etc. Also, in most such studies, the findings are at best applicable to considerable masses of cells mixed with other elements.

Many problems of neural function cry for more precise knowledge of the respiratory intensity per neurone or even neurone part. The present study, based on parallel measurement of oxygen consumption and cytoarchitecture of small bits of frog brain, is a step towards securing such knowledge.

METHODS. 1. *Tissue.* A frog was decapitated, the sides of the head snipped off, and the top of the brain case removed with forceps. The brain was carefully lifted on to a paraffin plate under a dissecting microscope and the meninges peeled off. Injured brains were discarded. A slice including the desired region was obtained by transections anterior and posterior to it, and then the particular region was cut out. A minimum of Ringer's solution (no substrate) was used. This was blotted off before weighing the tissue bit, which was remoistened in the capillary. Variations in QO_2 values (per gram moist tissue) are partly due to the difficulty of obtaining constant blotting, and proper weights, of tissue bits weighing but a milligram or two. From decapitation to completed dissection was never as much as five minutes, and respiration readings could be begun by another 5 to 10 minutes.

The structures chosen were of widely varied origin and architecture—the Anterior Olfactory Nucleus (AON), the Hippocampus (H), the Primordium Palii (PP), the Ventral Hypothalamus (VT), and the Cerebellum (C) (fig. 1). AON composes the dorsal half of a slice obtained by a section at the junction of the olfactory bulbs and hemispheres and one a millimeter caudal. The cells in this nucleus receive fibers from the olfactory tract and send processes to the hemisphere. H constitutes the dorso-medial segment of the ring-shaped slice

[1] A preliminary report of this work appeared in This Journal, **129**: 445, 1940.

obtained from the middle third of the hemisphere. It is sharply delimited by gross landmarks, is large and relatively homogeneous, and is obtained with minimal sampling error. It represents the most highly organized brain region in the frog. PP is lateral to H, on the crest and lateral wall of the hemisphere with superficial white matter and a periventricular cell sheet. Since samples were often obtained by a simple horizontal slice from the top of the hemisphere, when speed was at a premium, this region was less precisely dissected than the others. VT bulges out from the ventral brain surface, the pituitary having been removed with the meninges. It is easily cut from a slice which includes the hypothalamus and contains considerable white as well as grey matter. C is the dorsal portion of the slice taken between the optic lobes and the 4th ventricle, and contains about half white matter. Since C is largely separate in situ, a minimum of damage is produced by cutting in this case.

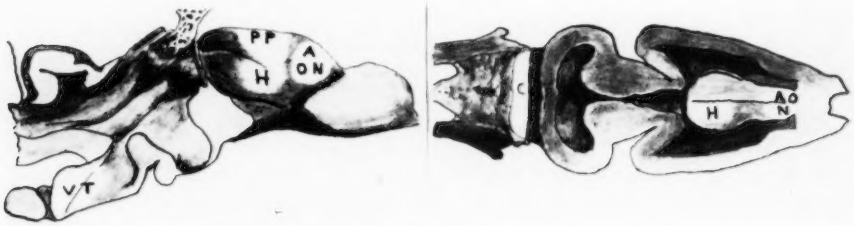


Fig. 1. Medial view of sagittal and view from above of horizontal section of the frog's brain, indicating the regions studied. Modified from "Handbuch der vergleichenden Anatomie der Wirbeltiere" and from Kappers-Huber-Crosby, Comparative Neurology, respectively.

2. *Respirometer.* The capillary respirometer (Gerard and Hartline, 1934) was used, with some modification, to measure oxygen consumption. To hasten temperature equilibrium, the instrument was placed vertically and the outer container filled with Ringer's solution except for 20 cc. of air trapped under inverted smaller tubes in it. Two thin-walled capillaries, about 2 sq. mm. cross section (later measured exactly in each case) and 200 c. mm. total volume, were carried on a lucite rod, which passed through the stopper of the outer vessel. Their upper ends were sealed with paraffin after inserting two filter paper strips and wetting them with isotonic KOH and H_2SO_4 respectively; tissue on a cellophane tray was placed through the other end of one, the second served as a control. All parts of the instrument were brought to temperature (24°C) in the thermostat before the capillaries were mounted, and then placing the rod and stopper forced Ringer's solution a few millimeters into the capillaries, to serve as index fluid. Movements of the menisci, illuminated from behind with green light, were followed with an ocular micrometer in a long focus microscope. Some sticking of the menisci occurred and vibrating the holder with a buzzer for 5 sec. before each reading improved the regularity of readings.

One micrometer division corresponded to a volume of 0.0016 cu. mm., but ir-

regularities rendered short interval readings of little value. (See, however, further improvements by Tobias and Gerard, 1941.) Control and experimental tubes were read at alternate half minutes, and a sliding 3 min. average of the control was subtracted from a similar average of the tissue tube. In obtaining absolute QO_2 values for each tissue type, the final 30 min. readings of each run were averaged.

3. *Evaluation of cells.* Respiration values per cell are so far available only on naturally separate cell species, such as blood or sex cells or unicellulars. Counts on histological sections are usually impracticable because of irregular distribution and large numbers—about 100,000 per mgm. of brain. A possible solution is to macerate the brain sample after determining its respiration, and to count and measure the cell nuclei thus brought into homogeneous suspension.

The older histologists made frequent use of macerating solutions. Recently Crossman (1937) (also Stoneberg, 1939) has used 5 per cent citric acid to isolate muscle nuclei, and Isaacs (1937) and Farrar (1936) have removed connective tissue from bone marrow with serum. Zirkle (1928, et. seq.) has shown that, while basic fixatives destroy nuclear structures, acid ones preserve the nuclear chromatin. This is especially true of acetic acid which, because of rapid penetration, dominates in any mixture containing it. Further, he found that acid, per se, does not affect the size of cell nuclei, the usual shrinkage being due to dehydration.

In the present experiments the tissue bit was taken from the capillary, usually one hour after the decapitation, and gently triturated for 2 min. (in a 5 cc. glass test tube with a glass pestle ground to fit) in a measured volume—at least 20 times the tissue mass—of 25 per cent acetic acid in water. This dissolved the cytoplasm but left the nuclei intact in suspension. The suspension was further diluted with nine volumes of a 0.1 per cent solution of gentian violet in distilled water, to stain the nuclei. After thorough mixing, the diluted suspension was drawn into a red cell diluting pipette, mixed for an additional two minutes, and a drop placed in the hemocytometer counting chamber for counting. Two hundred to 1000 cells were counted in each sample. In calculating the cells per milligram (cells per cubic millimeter in the final suspension, times total volume in centimeters of acid and dye solutions, divided by sample weight in milligrams), the volume of the brain sample was neglected.

Several possible sources of error have been explored. That maceration and mixing were adequate was shown by the reproducibility of cell counts on separate drops of the same tissue suspension. Independent duplicate counts, two on each of three brain regions, gave a maximum variation between two drops of 7 per cent, an average of 4 per cent. A more severe check, testing in part the maceration and nuclear destruction as well as the reproducibility of the entire procedures of suspension and counting, was to compare cell counts on bilateral brain samples. Tests on three brain regions gave differences of 4, 17 and 21 per cent —well within the error of exact dissection and weighing of the tissue bits.

Further tests were made with frog's nucleated red cells, by comparing nuclear counts with each other and with cell counts made with Hayem's solution as

diluent. Blood was diluted 22 times with acetic acid (in the white cell counting pipette), the liquid expelled and "ground" for 2 min., diluted a further 10 times with gentian violet, and enumerated. Another sample of the same blood was merely diluted twice (1:22 and 1:10) with Hayem's solution. In three tests, on different bloods, the counts were, in thousands per cu. mm., 549 and 560, 582 and 596, and 749 and 741 in acetic acid and Hayem's solution respectively—an average deviation of 1.7 per cent. When one blood sample was directly diluted (1:200) with Hayem's solution and another portion weighed, "ground" with acetic acid, etc., the counts differed by as much as 14 per cent. These results prove that red cell nuclei are not destroyed by the maceration procedure used and that, except for the weighing of the sample, the whole method is valid to within 5 per cent. Since counts on bilateral regions agreed as well as the weigh-

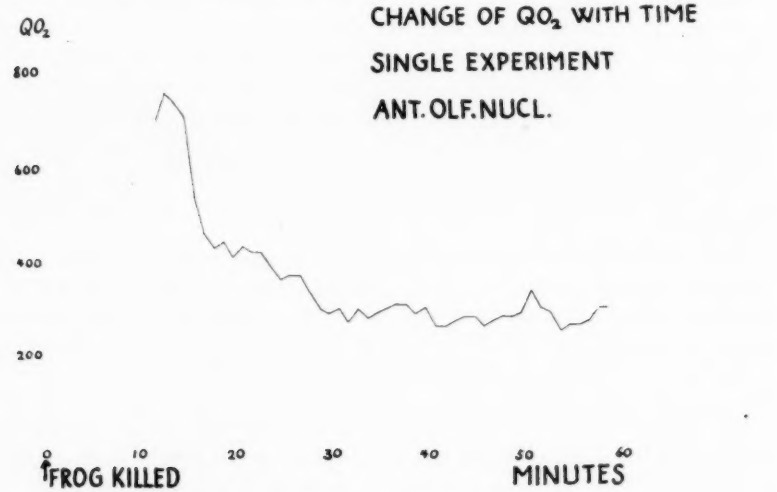


Fig. 2. Single experiment, illustrating reading variability

ing error permits, it is almost certain that no significant nuclear destruction occurred in these cells either.

The problem of what cells—neurones, neuroglia, endothelium, erythrocytes—are represented by the nuclei counted, and the related one of particular sizes and shapes of the nuclei from particular brain regions, will be considered later.

Results. 1. *Respiration.* Seventy-seven oxygen consumption curves were obtained from the 5 brain regions and the sciatic nerve. Figure 2 reproduces a single curve and table 1 gives average values for each tissue. Decapitation is taken as zero time in all curves and averages. All brain regions, but not the sciatic, showed initially rising values, to a maximum at 15 to 20 min., and then a decelerating fall for the remainder of the hour.[2] For all quantitative com-

[2] The initially low readings are the result of an instrumental error, perhaps an initial lag in CO_2 absorption. When one bilateral brain bit is mounted in a capillary as usual, and the other after standing for 20 min. or more on the cellophane tray, the second shows the same

parisons, the average value of the fairly stable respiration of the last half-hour was used. This was but little greater than the value for the final 5 min.

The respiration of frog nerve (Gerard, 1930) and brain (Rosenberg, 1935) averages over 60 per cent higher in summer animals than in winter ones. Similar differences appeared in these experiments, although most of them were performed in the winter. The results with PP, however, were mostly obtained in the summer and cannot be directly compared with other values.

Results are summarized in table 1, which also includes the statistical estimate of their quantitative reliability. Winter values for brain QO_2 range from about 560 to 730 at the maximum and 390 to 470 in the final half-hour, about 8 times that of the sciatic; and summer values are higher. Yet even these are con-

TABLE 1

Mean respirations of various regions of frog nervous system

TISSUE	NUMBER OF FROGS USED	MEAN QO_2* AT MAXIMUM	MEAN QO_2 FOR LAST 5 MIN. (1 HR. AFTER DECAPITATION)	MEAN QO_2 FOR LAST 30 MIN. AFTER DECAPITATION	σx† (FOR LAST 30 MIN.)	$\sigma \bar{x}$‡ (FOR LAST 30 MIN.)
Hippocampus	10	680	440	475	±115	±7
Cerebellum	10	725	355	400	±130	±8
Ant. Olf. Nucl.	10	685	350	390	±165	±7
Hypothalamus	6	565	370	395	±155	±12
Sciatic nerve	10	90	60	65	±30	±2
Primordium Pallii	10	1055	610	675	±735§	±50

* QO_2 = mm^3 O_2/hr/gm wet weight.

† σx (standard deviation of the readings) = $\sqrt{\frac{\Sigma (x - \bar{x})^2}{N}}$, where x is a single reading, $\bar{x}$ the mean, N the number of readings. Over two-thirds of the readings normally fall in the range, $\bar{x} \pm \sigma x$.

‡ $\sigma \bar{x}$ (standard error of the mean) = $\frac{\sigma x}{\sqrt{N}}$. The chances of the observed $\bar{x}$ being more than $3\sigma \bar{x}$ from its "true" value are less than 3 in 1000.

§ The great variability of primordium pallii readings is due to sampling conditions. In many of these experiments a very rapid removal was the prime consideration and constant anatomical bits were not obtained.

low initial readings as had the first. Thus, in seven paired tests on PP (about 1.5 mgm. each) readings on the first samples averaged 1000 9.5 min. after decapitation, rose to 1400 4 min. later, and had fallen to 765 at 25 min. The second samples were mounted after half an hour and readings averaged 450 9 min. later, rose to 740 in another 5 min., and had fallen to 520 at the end of the run, one hour from decapitation. Clearly, except for the initial low period, the respiration of the second sample is a continuation of that of the first. The maximum values may, correspondingly, be somewhat excessive due to an "overshoot." This seems unlikely in view of the paired runs discussed and of the extensive evidence of a progressive fall of brain respiration after isolation (see Gerard, 1938), but is another reason for our using the later respiration values for quantitative evaluation. It is also worth noting that the high values during the first half-hour prove that oxygen diffusion into the tissue is not a limiting factor. This was further tested by the use of larger brain bits, which gave the same QO_2 values as smaller ones.

siderably above those of Rosenberg (1935), in the range 150 to 300, and of Ashford and Holmes (1931), 330 for intact and 250 for chopped brain; they are of the same order as those of Bass (1923), 415. Spring values for these same brain regions, obtained under similar but not identical conditions (Tobias and Gerard, 1941) average some 20 per cent higher. The maximal values here reported are probably more indicative of the *in vivo* rates than are the later ones; the lower values of other workers may be due to their working later after dissection and with whole brain, which contains a higher proportion of white matter than do our regions. The early high values are not due to injury—compare C, with only one cut surface, to AON with four—nor to an oxygen debt, for any possible inadequacy of oxygen could have lasted 3 to 5 min. at most. Conversely, the later low values are to be expected from substrate depletion and possible loss of a serum factor (Brookens, Ectors, and Gerard, 1936; Schaffer, Chang, and Gerard, 1935).

H has a higher respiration rate than the other brain regions, and is to be regarded as a "higher" center. Other direct *in vitro* respiration measurements on pigeon (Gavrilescu and Peters, 1931) and ox (Dixon and Meyer, 1936) (see also Quastel, 1939) offer interesting comparisons. The QO_2 of pigeon cerebellum, 1030, is 2.60 times that of the frog; and the cerebrum QO_2, 1260, is 2.65 times that of the frog's hippocampus. Ox cerebral cortex, 1700, ammon's horn, 1260, and thalamus, 1170, QO_2 values are respectively 3.6, 2.7, and 2.9 times as great as those of the most equivalent frog structures. Evidence from anoxia (Sugar and Gerard, 1938; Hermann, et al., 1939) and hypoglycemia (Tyler and Ziskind, 1940) also indicates a parallelism between metabolic intensity of a brain region and its position along the neuraxis and in evolution.

2. *Cell population.* Figure 3 reproduces photomicrographs of characteristic fields obtained after maceration and staining of each of the brain regions. Although some debris is always present, the nuclei stand out as stained units containing chromatin structures and with a smooth unbroken boundary, the nuclear membrane, clearly visible. Only these were counted and measured; and the absence, or constancy, of any personal factor of selection is indicated by the degree of statistical reliability of the results.

It is apparent at once from figure 3 that the cell populations of the different brain regions are fairly unique for each, as judged by nuclear sizes and numbers. Similarly, the average cell count per hemocytometer square (0.1 cmm.) was 105 for C, 70 for AON, and 27 for H. The results of the cell counts are presented in table 2. Despite considerable variation from brain to brain, the count for each region centers around its own characteristic value, which is significantly different from that of others.

For a more exact analysis, and to distinguish between kinds of cells, nuclear dimensions were measured with an ocular micrometer. The greatest diameter and the maximum width at right angles to this were obtained on 100 consecutive nuclei, as located across the counting chamber, of each preparation. Since 10 such preparations of AON, H, and C were studied, a composite picture of 1000 nuclei was obtained for each of these regions.

Nuclei of blood cells were obtained by applying the maceration technique to whole blood, and the dimensions of 300 nuclei, from 3 samples, measured as above. To identify endothelial cell nuclei in the brain bits, the trituration in acid was stopped early, in half a minute, and the imperfectly macerated tissue then stained as usual. Scraps of capillary endothelium were thus preserved and their nuclei identified. Three hundred of these were measured. The dis-

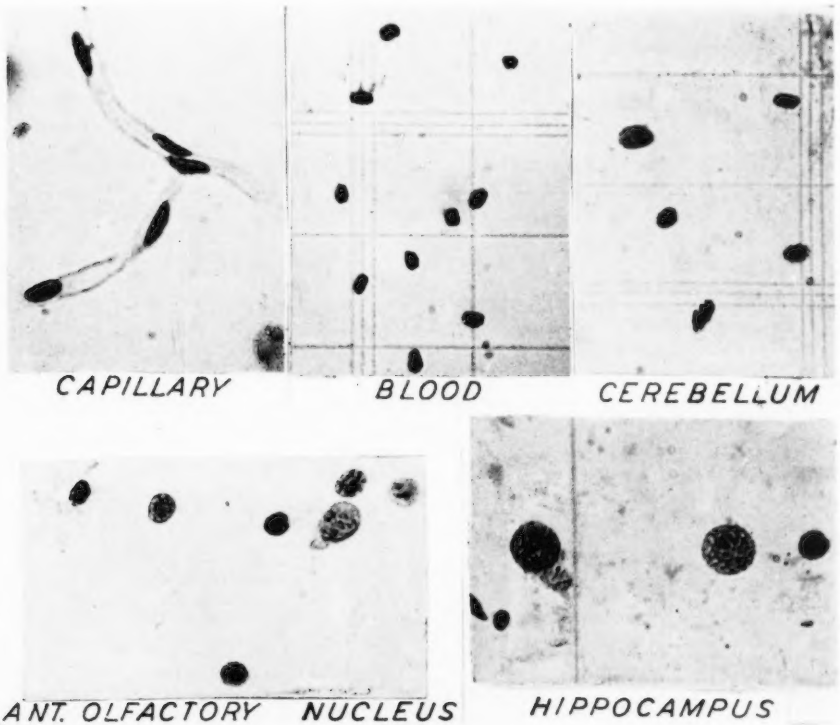


Fig. 3. Photomicrographs of characteristic fields obtained after maceration and staining of nuclei. Five-hundredths millimeter between squares on the R.B.C. field gives magnification.

crimination of glial and neurone nuclei was not satisfactory.[3] These cell types will, therefore, be considered together.

Although fixation shrinkage and distortion would not affect relative measurements of the different cell nuclei, absolute values might be seriously off. To check this, red cell nuclei were measured after treatment with Hayem's solution and with acetic-gentian violet and the results compared. Table 3 shows clearly

[3] Using toluidine blue, instead of gentian violet, Doctor Tobias finds the glial nuclei to stain with a more greenish tinge than those of neurones, but the conditions for obtaining this differentiation have not been worked out.

TABLE 2

Total cells per milligram for each tissue (multiply all figures by 10^3)

EXPERIMENT NUMBER	BLOOD	HIPPOCAMPUS*	CEREBELLUM	ANTERIOR OLFACTORY NUCLEUS	HYPOTHALAMUS	WHOLE CENTRAL NERVOUS SYSTEM
1	640	60	215	175	145	61
2	560	49	205	115	100	
3	550	46	250	190	175	
4	550	48	260	90	145	
5	580	49	235	90	155	
6	595	42	260	135		
7	750	55	315	75		
8	740	45	165	145		
9	690	62	200	105		
10	610	67	170	120		
$\bar{x}$	627	52	228	124	143	
σx	69	8	43	36	25	
$\sigma\bar{x}$	±26	±3	±16	±13	±14	

* The more consistent figures for hippocampus than for other regions are undoubtedly the consequence of better sampling. Because of the few large cells, more drops of suspension were counted. Had a similarly extensive sampling been used in the other cases a corresponding improvement in reliability should have resulted.

TABLE 3

Comparison of nuclear dimensions in maceration fluid and in Hayem's solution

	METHOD OF PREPARATION	
	Acid and gentian violet	Hayem's solution
	100 cells	50 cells
Surface:		
Average	144	150
σx	29	33
$\sigma\bar{x}$	±2.9	±4.6
P	0.12	
Volume:		
Average	135	140
σx	41	24
$\sigma\bar{x}$	±4.1	±3.4
P	1.4	

Statistical analysis of the measurements on red cell nuclei prepared in Hayem's and in acid and gentian violet. Surface was calculated, for convenience, by the formula of $S = \pi dl$, where d and l are the short and long diameters respectively. Volume is calculated by the formula $V = \frac{\pi}{6} d^2 l$. P is the probability that the two samples are from the same universe. As P is much greater than 0.05 this identity can be taken as established.

that nuclei prepared by both methods fall into a single population; that is, the two treatments are equivalent. This finding is in agreement with Zirkle's results.

A further test was made by comparing nuclear dimensions of bilateral bits from the same brain. In figure 4 the abscissa represents the long nuclear diameter, the ordinate the short one, and each nucleus is represented by a dash within the one micron square to which its measurements relegate it. Since nuclei from one side are shown as horizontal dashes and those from the other as

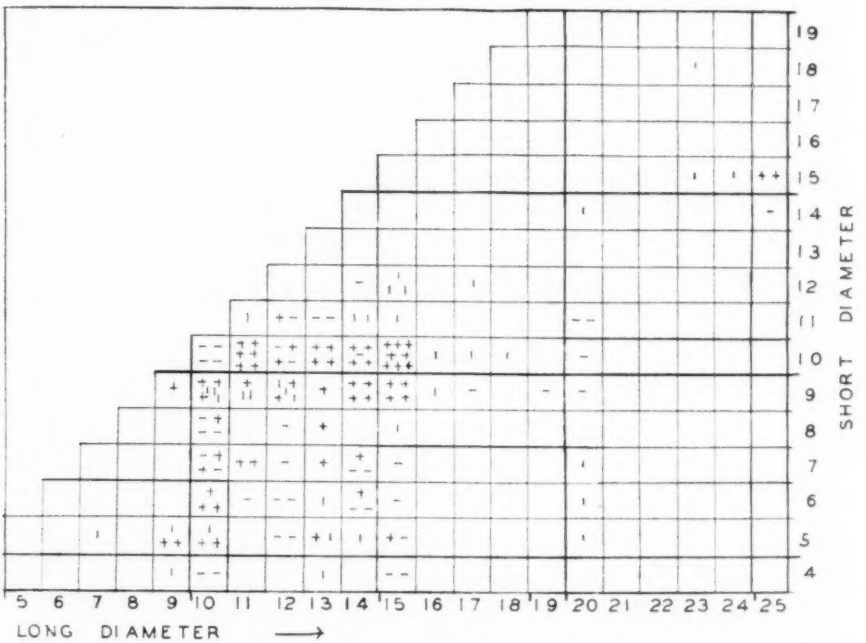


Fig. 4. Chart showing distribution of nuclear dimensions from like bilateral samples of one brain. The long and short diameters are indicated in microns. Each dash represents one nucleus; horizontal ones from one side of the brain, vertical ones from the other.

vertical ones, the crosses at once indicate the high degree of coincidence of the two sets. A similar amount of overlap was obtained when some nuclei in a given suspension were measured at once after preparation and others 24 hours later. Finally, measurements made on nuclei in fixed (formol acetic) and stained frog brain sections were compared with those on macerated preparations (we are indebted to Dr. M. L. Silver for values on one such set).

Rough long and short nuclear diameters, in sections and suspension respectively, are: red cells, 8 x 4 and 10 x 5; endothelial cells, 12 x 8 and 15 x 7; H, 12 x 12 and 17 x 17; C, 8 x 6 and 10 x 10; AON 10 x 6 and 15 x 10. Blood and

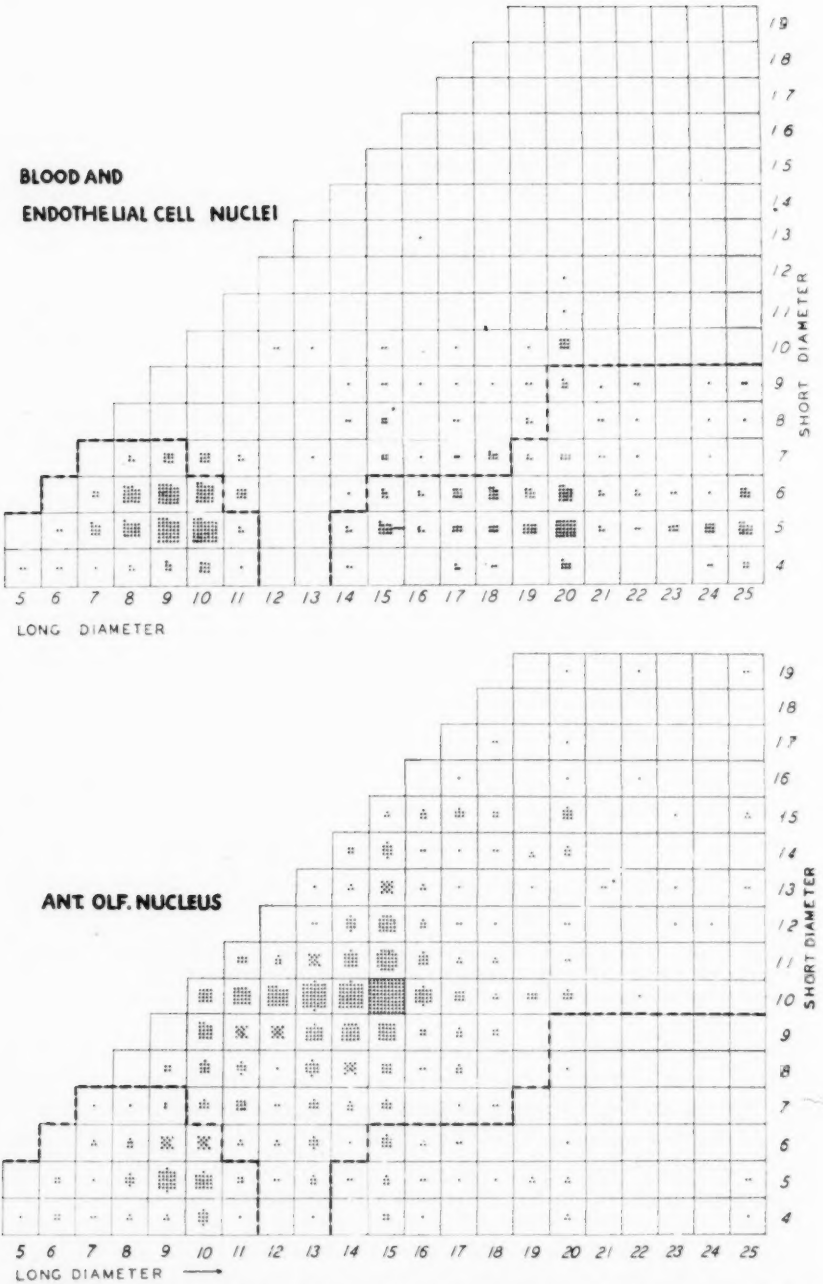
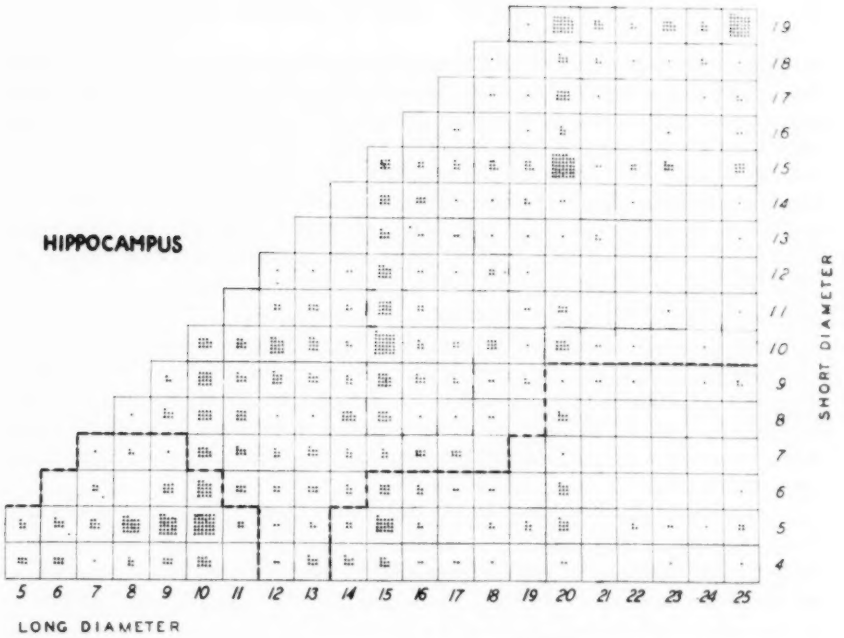


Fig. 5. Charts showing the distribution of nuclear dimensions in various tissues. Each dot represents one cell nucleus of the indicated dimensions in microns. The blood and endothelial cells were measured in separate preparations. The dotted lines in the brain charts are drawn to include 90 per cent of these non-neural cells.


HIPPOCAMPUS
SHORT DIAMETER
LONG DIAMETER
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4

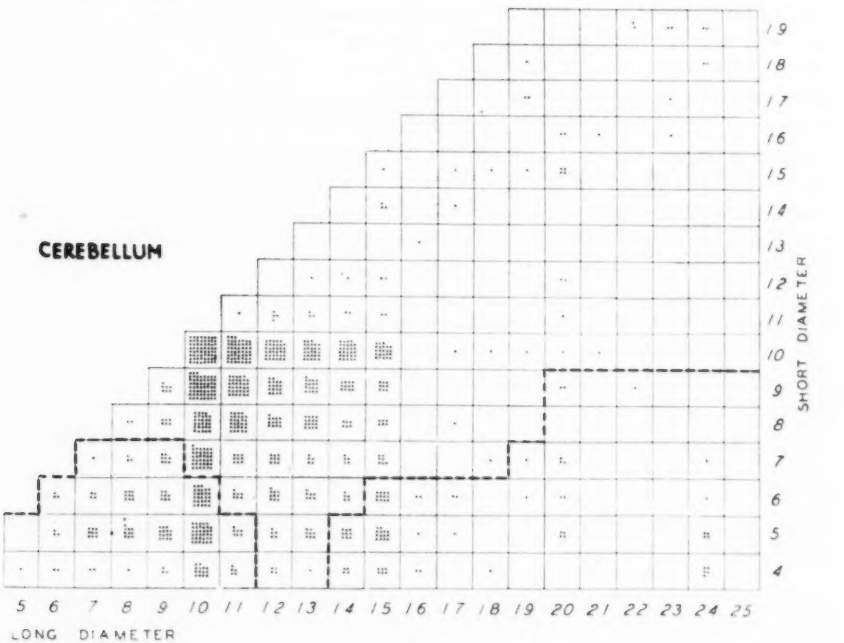

CEREBELLUM
SHORT DIAMETER
LONG DIAMETER
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4

Fig. 5

capillary cell nuclei show fair agreement, the fixed preparations being somewhat larger, while the discrepancy with nuclei of neural elements is greater. Since technical errors also appear in section measurements and since a large sampling error is probable (only a few dozen nuclei were measured), it is hard to evaluate this divergence; but obviously the absolute dimensions we have used are open to some question. For a doubling of radii, values per unit surface would be low in absolute units by a factor of four; values per unit volume, by a factor of eight. Fortunately, even if all neural nuclei are swollen in our suspensions, the various relationships will still hold, as between the different brain regions, if the swelling be proportional to true size—a reasonable assumption.

Charts showing the distribution of nuclear dimensions for the three intensively studied brain regions and for erythrocytes and endothelial cells are presented in figure 5. That these differ is patent. Cerebellar nuclei are small and rather homogeneous, centering about 18 x 10 mμ; those of AON are more variable and center about 10 x 15 mμ; while those of H are quite scattered and run to much larger dimensions.

It is necessary to correct the brain regions by excluding blood and endothelial nuclei in order to deal only with neural elements. This is difficult because of some overlap in dimensions of nuclei of these different types; but it is none-the-less possible to make an adequate separation. An area was blocked off on the nuclear dimension charts of blood and of endothelial cells so as to include 90 per cent of all nuclei in each case. The same areas were then marked on the charts for each neural region and the nuclei in them excluded from the final count. Thus, presumably, the neural count includes some 10 per cent each of the other cell types and excludes an unknown portion of neural elements. Actually, the overlap of dimensions is small enough and the percentage of non-neural nuclei in the suspension low enough so that varying the areas subtracted, to include 100 per cent or 80 per cent of the non-neural nuclei instead of the usual 90 per cent, alters the neural cell count by only 2 to 3 per cent. The error in evaluating blood and endothelial cells is, of course, greater. Further refinements should, however, make it possible to estimate the relative capillary length and the blood supply[4] for each brain region from such nuclear studies. Table 4 gives the cell relations as obtained by our present procedure.

3. *Nuclear dimensions.* Aside from their value in distinguishing cell types, the nuclear diameters permit calculation of other nuclear dimensions. Assuming an ellipsoid shape of the nucleus, the third, unmeasured, diameter was taken equal to the shorter measured one. Nuclear volume was then obtained from the formula $V = \frac{\pi}{6} d^2 l$ (d = short diameters, l = long one). Nuclear surface was calculated, for convenience, in terms of a cylinder, of length $l - d$, capped by hemispheres, of diameter d. $S = \pi dl$. For other comparisons, nuclear volume

[4] For example, from the known red cell count and the average number of red cell nuclei per milligram of brain tissue, it can be calculated that blood constitutes over 2 per cent of the brain volume, despite the free hemorrhage permitted on decapitation. Compare with the value of 8.3 per cent for the dog's brain (Weil, Zeiss and Cleveland, 1931).

squared (V^2) and to the three halves power ($V^{3/2}$) have also been calculated. These four values were calculated for each set of nuclear dimensions. To obtain, say, total nuclear volume, ΣV, per cubic millimeter of any brain region, the

TABLE 4

Percentage and absolute numbers per milligram of red blood cells, capillary cells, and nerve and glia cells in each tissue. (Calculated from fig. 5)

TISSUE	NERVE AND GLIA CELLS	RED BLOOD CELLS	CAPILLARY CELLS	TOTAL CELLS PER MGM.	RED BLOOD CELLS PER MGM.	CAPILLARY CELLS PER MGM.	NERVE AND GLIA CELLS PER MGM.
	per cent	*per cent*	*per cent*				
Hippocampus	67	17.5	15.5	52,300	9,000	8,000	35,000
Ant. Olf. Nuc.	80.5	13	6.5	123,400	15,000	8,000	100,000
Cerebellum	70	19	11	227,600	43,500	25,000	160,000

TABLE 5

Calculated functions of the nuclear dimensions of nerve and glia cells

FUNCTION	CELLS	HIPPOCAMPUS	CEREBELLUM	ANT. OLF. NUCL.
Average function per cell, in microns				
Nuclear surface (μ^2)	Total	405 ± 13*	275 ± 4	380 ± 4
	"Nerve"	505	315	420
Nuclear volume (μ^3)	Total	935 ± 45	395 ± 14	585 ± 14
	"Nerve"	1290	495	685
(Nuclear volume)^2 (μ^3)^2	Total	2,950,000 ± 210,000	325,000 ± 40,000	539,000 ± 35,000
	"Nerve"	4,350,000	445,000	654,000
(Nuclear volume)$^{\frac{3}{2}}$ (μ^3)$^{\frac{3}{2}}$	Total	45,500 ± 2,900	10,000 ± 720	17,000 ± 700
	"Nerve"	66,000	13,500	20,000
Average function per milligram of tissue, in microns ($\times 10^{-6}$)				
Nuclear surface	Total	21.5 ± 1.4	62.5 ± 4.5	46.5 ± 5.0
	"Nerve"	17.5	50.5	41
Nuclear volume	Total	48.5 ± 3.6	89.5 ± 7.0	71.5 ± 7.9
	"Nerve"	45	78.5	68.5
(Nuclear volume)^2	Total	155,000 ± 14,000	74,000 ± 11,000	66,000 ± 8,000
	"Nerve"	155,000	71,000	65,500
(Nuclear volume)$^{\frac{3}{2}}$	Total	2,400 ± 200	2,300 ± 230	2,100 ± 240
	"Nerve"	2,300	2,200	2,000

* These represent the standard errors of the means. For the average function per cell for each tissue these were calculated as described in table 1. For the total surface, volume, etc. per milligram the standard errors were calculated by the formula for a product. For example, if the average nuclear volume per cell in a given tissue is $V \pm \sigma\overline{V}$, and the number of cells per milligram is $N \pm \sigma N$, the average nuclear volume per milligram is

$$NV \pm \sigma\overline{NV}, \quad \sigma\overline{NV} = \sqrt{V^2(\sigma\overline{N})^2 + N^2(\sigma\overline{V})^2}$$

volumes corresponding to nuclei in each dimension group were summed in numbers proportional to (by the factor, total nuclei per cubic millimeter over 1000) the nuclei found in that dimension area in the plot (fig. 5) of the desired

brain region. The average nuclear volume was then obtained by dividing this total volume per cubic millimeter by the cells per cubic millimeter. Such total and average nuclear functions were calculated, for each of the brain regions intensively studied, on the basis of all cells (1000 for each region) measured in ten experiments. Similar calculations were also made for the neural elements only, after excluding blood and capillary cells. The results are given in table 5.

Discussion. Bok (1936) has published exhaustive quantitative studies of the neurone population of several areas of the human cortex, supplemented by some on primate cord (1934). He reports many striking relationships between nuclear parameters and other cell dimensions and distributions. Thus, for various cytoarchitectonic regions, with few large or many small neurones, total nuclear volume squared (ΣV^2, the sum of V^2 of all individual nuclei) is constant. Strictly proportional to V^2 is the cytoplasmic mass of each neurone (the perikaryon volume, equal to the volume of cell body after excluding nucleus and processes); and also the "territory" governed by each neurone—the volume occupied by its dendrites and by the terminals of other cells connecting with these—which is 35 times the perikaryon volume. The total length of all nerve processes is constant per unit brain volume, and so proportional to ΣV^2; and the dendrite length of each neurone varies as its own V^2, so that total dendrite length is also constant per unit brain volume. Thus, all brain regions have a constant proportion of perikarya, dendrites, and incoming fibers, but each is characterized by the size of the individual cell units—and all these quantities vary directly as V^2 or ΣV^2.

The surface area of the perikaryon is also a function of its nuclear volume, but in this case directly proportional to V; whereas the total volume of each cell body (nucleus and cytoplasm) is proportional to $V^{3/2}$. Thus, per unit volume of grey matter, cytoplasmic mass, dendrite length, and total nerve fiber length vary as ΣV^2; perikaryon surface varies as ΣV; and protoplasmic volume as $\Sigma V^{3/2}$.

This study covers three widely differing brain regions in the frog, each extending from pia to ventricle and so presumably including the full "territories" of the contained neurones. An inverse relation between size and number of cells in these regions is at once apparent, but ΣV^2 is not a constant. Either, therefore, such attributes as cytoplasmic mass and dendrite length are not proportional to V^2 in frog neurones or their total values are not constant per brain mass. ΣV, and presumably perikaryon surface, is also not constant from one brain region to another. $\Sigma V^{3/2}$ is, however, fairly constant in these frog brain regions and so, perhaps, is the volume of cell protoplasm.

Interestingly, the fraction of brain volume occupied by cell bodies decreases—and that available for synaptic field increases—from frog to man. Thus, Donaldson (1911) estimated cell bodies to occupy 2 per cent of the human brain and 3 to 5 per cent of the rat brain, and Bok finds almost 3 per cent of human cortex occupied by cell cytoplasm; while we find 6 to 8 per cent occupied by nuclei alone in the frog. Truszkowski's finding (1928), that the ratio of purine to total nitrogen is 50 per cent higher in frogs than in mammals, suggests a similarly greater nuclear mass in frog tissues as a whole.

Before correlating the respiration data with the cytological findings, some consideration of the oxygen consumption of non-neural cells is necessary. Measurements of the respiration of frog blood gave an average QO_2 of 36; and of urostyle bone marrow (as somewhat representative of endothelial elements) a QO_2 of 74 for a preparation with 1.6×10^5 cells per milligram. The average brain sample contained 0.03 mgm. of blood and 10^4 endothelial cells per milligram. These would thus account, respectively, for 1 and 5 cm. O_2 per hour per gram of brain—entirely negligible amounts in comparison with the measured QO_2 values of 400 to 500. It is also to be noted that, since respiration and cell measurements are available on the same brain bits, weighing and other sampling errors will cancel rather than cumulate when oxygen consumption is calculated in terms of cells rather than of total mass.

TABLE 6

Respiration as a function of the parameters of the cell nuclei of each brain region

FUNCTION	CELLS	HIPPOCAMPUS	CEREBELLUM	ANT. OLF. NUC.
QO_2/cu.mm.O_2/gm./hr.		475 ± 7	400 ± 8	390 ± 7
O_2 uptake per cell (cu.mm. per hr. $\times 10^{-6}$)	Total	9.1	1.7	3.1
	"Nerve"	13.5	2.5	3.9
QO_2 per unit surface (cu.mm.O_2/μ^2/hr. $\times 10^{-3}$)	Total	22.3 ± 1.8*	6.4 ± 0.5	8.3 ± 0.9
	"Nerve"	26.8	7.9	9.3
QO_2 per unit volume (cu.mm.O_2/μ^3/hr. $\times 10^{-9}$)	Total	9.7 ± 0.7	4.5 ± 0.4	5.4 ± 0.6
	"Nerve"	10.5	5.1	5.7
QO_2 per unit (volume)^{2} (cu.mm.$O_2/(\mu^3)^2$/hr. $\times 10^{-12}$)	Total	3.1 ± 0.3	5.4 ± 0.8	5.8 ± 0.7
	"Nerve"	3.1	5.6	6.0
QO_2 per unit (volume)$^{\frac{2}{3}}$ (cu.mm.$O_2/(\mu^3)^{\frac{2}{3}}$/hr. $\times 10^{-12}$)	Total	200 ± 17	175 ± 18	185 ± 22
	"Nerve"	205	195	195

* The standard error, of the respiration per particular tissue dimension, is calculated on the basis of the statistics of the component variables by a formula for the standard error of ratios (see J. B. Scarborough, *Numerical Mathematical Analysis*, The Johns Hopkins Press, Baltimore, 1930).

$$\sigma\left(\frac{\bar{x}}{\bar{y}\bar{z}}\right) = \frac{\bar{x}}{\bar{y}\bar{z}}\sqrt{\left(\frac{\sigma\bar{x}}{\bar{x}}\right)^2 + \left(\frac{\sigma\bar{y}}{\bar{y}}\right)^2 + \left(\frac{\sigma z}{\bar{z}}\right)^2}$$

Where $\bar{x}$, $\bar{y}$ and $\bar{z}$ are the means respectively for respiration, cell number, and nuclear volume. We are indebted to Miss Eleanor Gish for assistance with all of the statistical calculations in this paper.

Table 6 gives the respiration as a function of various cell parameters for each brain region. It is obvious at a glance that, while respiration per milligram is but little greater in H than in AON or C, respiration per cell is 3 to 5 times as great—H having few large cells—while respiration per mass of cell protoplasm ($\Sigma V^{2/3}$) is essentially alike in all. Further study shows that, assuming Bok's relations, the respirations of different brain masses are alike only when expressed as a function of the total nerve cell protoplasm contained in each, and not when correlated with the summated: volumes of nuclei or of cytoplasms alone, surfaces of nuclei or of cell bodies, lengths or surfaces of cell processes. Thus these findings, somewhat disappointingly, point to the whole protoplasmic mass rather than to surface membranes as determining the magnitude of the resting metabolism and the energy requirement of neurones.

SUMMARY

1. Bits of frog brain tissue, about 1 mgm., were dissected from widely different structural regions: anterior olfactory nucleus (AON), primordium pallii (PP), hippocampus (H), ventral hypothalamus (VT), and cerebellum (C).

2. Respiration of these bits, as of nerve, was determined in a capillary respirometer; and the tissue was then macerated and the nuclei of nerve cells (and glia, capillary endothelium, and red blood cells) counted and measured. The method of cell evaluation is intrinsically valid to 5 per cent.

3. QO_2 values, up to 700, are higher than most of those previously reported for frog and average about 40 per cent of those for mammalian brain. In order of descending QO_2, the series is C, H, AON, (PP), VT at first; with C falling off faster than other regions.

4. Nerve cells per milligram vary from 35,000 large neurones in H to 160,000 small ones in C, with a characteristic size population in each region. The total cell volume is roughly the same for all regions.

5. These data make it possible to express oxygen consumption as a function of the total neurones and, assuming the morphologic relations described by Bok, of the total neurite surface, perikaryon surface, process surface, nuclear surface, nuclear volume, perikaryon volume and total protoplasm volume. All these functions are analyzed statistically for valid correlations.

6. The respiration of various brain regions is constantly related to the mass of protoplasm, but not to the surface area, of the neural elements present.

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OXIDASES, PRESSOR AMINES AND HYPERTENSION[1]

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According to Schroeder (1), high blood pressure can be treated with tyrosinase. The available enzyme preparations were found to be effective only when injected in large quantities, frequently resulting in toxic manifestations (1), typical for foreign proteins. Therefore, tyrosinase therapy of hypertension would be greatly improved if the dosage could be reduced considerably. It is conceivable that compounds could be found which facilitate or accelerate in vitro the enzymatic destruction of typical phenolic pressor amines, and which accelerate the action of tyrosinase in the hypertensive animal. The use of such a compound in conjunction with tyrosinase will probably permit the administration of smaller doses of the enzyme, thus reducing the possibility of anaphylactic reactions. Therefore, we determined the influence of various substances on the aerobic oxidation of adrenaline, a typical pressor amine, by tyrosinase. Moreover, since dopa is considered to be the precursor of the pressor amine arising in the kidney of hypertensive animals, the effect of substances found to alter the oxidative degradation of adrenaline by tyrosinase was investigated in dopa-kidney tissue systems.

EXPERIMENTAL. *Experiments with adrenaline-tyrosinase.* The in vitro enzymatic experimental procedure consisted in permitting the enzyme tyrosinase[2] to act at pH 7.1 in a medium composed of 2.5 ml. of a saline solution, 0.5 ml. of a 1:1000 dilution of adrenaline, and 2.0 ml. of a phosphate buffer solution. The test compounds were usually added in 1 mgm. amounts to this reaction mixture. After agitation for exactly twenty minutes at incubator temperature, a 2.0 ml. aliquot was immediately administered intravenously to cats or dogs and the effect on blood pressure observed. Under these experimental conditions, 2.0 ml. of a control solution, i.e., one which had been incubated in the absence of a test substance, was found to give an average pressor rise of 60 mm. of mercury. This response is the same as that obtained from the intravenous injection of 0.1 ml. of 1:10,000 adrenaline. With catechol as the test compound added in amounts varying from 0.001 to 1.0 mgm., no change in blood pressure was ob-

[1] Presented in part at Atlantic City, N. J. on September 11, 1941 (102nd Meet. Am. Chem. Soc., Div. Biol. Chem.).

[2] One-tenth milliliter of Stock Solution no. 2, prepared in the laboratory of Prof. J. M. Nelson of Columbia University, and containing approximately 1500 catechol-hydroquinone units (2) and 200 p-cresol units (3) per ml. measured at 25°C.

served, indicating a complete destruction of adrenaline during the incubation period. When p-aminobenzoic acid in 1 mgm. quantities was the test substance, a rise of 120 mm., i.e., 60 mm. more than the control solution, was observed. This increase is the same as that obtained from the intravenous injection of 0.5 ml. of 1:10,000 adrenaline. Therefore, p-aminobenzoic acid seems to cause an inhibition of the destruction of adrenaline by tyrosinase. Apparently, calcium pantothenate and other B-complex vitamins likewise at the 1 mgm. level had no influence, the blood pressure rise being the same as that obtained when the control solution was injected.

TABLE 1

Catalysis of adrenaline oxidation by tyrosinase

Column I		Column II		Column III	
SUBSTANCES WITH AN INHIBITORY EFFECT*		SUBSTANCES WITH NO EFFECT†		SUBSTANCES WITH AN ACCELERATING EFFECT*	
	x‡ *mgm.*		x‡ *mgm.*		x‡ *mgm.*
A				A	
Thiosalicylic Acid	0.01	Benzocaine	1.0	Catechol	0.01
p-Phenylenediamine	0.1	Calcium Pantothenate	1.0	o-Cresol	1.0
Aniline	1.0	Ferric Chloride	1.0	Aseptoform	1.0
Benzoic Acid	1.0	Hydroquinone	1.0	p-Hydroxybenzoic Acid	1.0
Orcinol	1.0	Inositol	1.0	Phenol	1.0
p-Aminobenzoic Acid	1.0	Nicotinic Acid	1.0	o-Aminophenol	0.1
m-Aminobenzoic Acid	1.0	Novocaine	1.0		
o-Aminobenzoic Acid	1.0	Pyridoxine HCl	1.0		
Pyrogallol	1.0	Sulfanilamide	1.0		
		Thiamine HCl	1.0		
B				B	
Ascorbic Acid	1.0			p-Hydroxyphenylacetic Acid	1.0
p-Nitrobenzoic Acid	1.0			Dopa	1.0
Ephedrine	1.0				

* In order of decreasing action.

† In alphabetical order.

‡ x is the amount of test substance added to the tyrosinase-adrenaline mixture which was incubated at 37°C. for 20 minutes before being intravenously injected into a dog or cat. The mixture incubated without the test substance gave a 60 mm. blood pressure rise. This increase is the same as that obtained from 0.1 ml. of 1:10,000 adrenaline. Mixtures incubated with the substances listed in column IA gave a 120 mm. rise (same as 0.5 ml. of 1:10,000 adrenaline), with those of column IB, 110 mm. (same as 0.4 ml. of 1:10,000 adrenaline), with those of column II, 60 mm., with those of column IIIA, no change in blood pressure, and with those of column IIIB, a slight increase (same as 0.05 ml. of 1:10,000 adrenaline).

The various substances tested are classified in table 1 according to their influence on the destruction of adrenaline by tyrosinase. Column 1 shows the compounds which had an inhibitory influence on adrenaline destruction, as evidenced by a rise of blood pressure above that obtained with the control solution. In column 2 are the substances which were found to be inactive, and column 3 lists the compounds with an accelerating effect, as seen from the fact that only a small, if any, pressor response was observed.

Experiments with dopa-kidney tissue. As pointed out in the introduction, the various test substances were further studied in order to determine whether they play a rôle in the formation of pressor amines by the kidney. For this purpose, 2 grams of minced kidney tissue from dog or cat was incubated at pH 7.1 for

about 18 hours in 30 ml. of a phosphate buffer solution containing 40 to 60 mgm. of dopa. The test compounds were added in concentrations of 1 to 10 mgm. per 30 ml. of the mixture to be incubated. The incubated product was centrifuged, and 2 to 10 ml. of the supernatant liquid was injected intravenously into a cat or a dog, and its effect on blood pressure recorded.

Under these experimental conditions it was found that substances increasing (table 1, column 3) the oxidation rate of adrenaline by tyrosinase, or decreasing (table 1, column 1) this rate, also influence in the same manner the production of pressor substances by kidney tissue acting on dopa. In other words, the experiments showed that there is a parallelism between the results in the dopa-kidney system and those of the adrenaline-tyrosinase studies already described.

Experiments with perinephritic-hypertensive dogs. Compounds which promote in vitro the destruction of adrenaline by tyrosinase (table 1, column 3) also facilitate the action of the latter in reducing blood pressure. It was found, namely, that 15,000 catechol-hydroquinone units of tyrosinase are required intramuscularly to reduce the blood pressure of the experimentally produced hypertensive dog from 230 to 150 mm., and that only 500 units of the same tyrosinase preparation are necessary when catechol is first administered at a level calculated to bring the concentration to 10γ per ml. of blood. Since dopa may act as catechol in catalysing the destruction of adrenaline, the experiments were repeated using dopa in place of catechol. An aqueous suspension of 100 mgm. of dopa was injected intramuscularly immediately preceding the intramuscular administration of 700 units of tyrosinase. The blood pressure of 230 mm. did not fall, as it did in the catechol experiments, but was further elevated. This result is seemingly not in accord with the data of table 1 showing a similarity of catechol and dopa in their effect on tyrosinase activity. However, it is in harmony with the results obtained in the dopa-kidney tissue studies already described. Apparently, dopa may be decarboxylated to produce a pressor amine which is hydroxytyramine, according to Bing (4).

Discussion. Ortho substituted phenols (e.g., catechol, cresol, aminophenol) and compounds such as dopa and p-hydroxybenzoic acid facilitate the enzymatic destruction of adrenaline. Catechol is particularly potent, in fact, even ten times more effective than when allowed to influence the non-enzymatic oxidation of adrenaline.

The three isomeric aminobenzoic acids and several structurally related substances inhibit the enzymatic destruction of adrenaline. Aminobenzoic acids were shown by Woolfe (5) not to protect adrenaline against non-enzymatic in vitro oxidation. These compounds do not destroy tyrosinase, nor are they themselves oxidised in preference. Therefore, their observed activity is probably due to enzyme blockage. An additional attribute may thus be added to the list of properties of p-aminobenzoic acid, i.e., inhibition of the enzymatic oxidation of adrenaline, already confirmed by the Warburg technique, the results of which showed a direct correlation between oxygen consumption (6) and those obtained in the present communication by pharmacological testing.

The study of adrenaline oxidation by tyrosinase brought out the fact that there exists a definite structural relationship among the catalysing benzene

derivatives investigated. Compounds with an amino group, carboxyl radical, or both, in ortho, meta, or para position on the ring, have an inhibitory action as, e.g., aniline, benzoic acid, and the isomeric aminobenzoic acids. The introduction of a hydroxyl radical into the inhibiting molecules of aniline and benzoic acid produces an aminophenol and a hydroxybenzoic acid, respectively, both of which were found to accelerate tyrosinase action, although by different processes. Structural relationship plays no rôle when protection is afforded to adrenaline by substances which do not block the enzyme as, e.g., ascorbic acid which is inherently oxidised with ease. Thiosalicylic acid is definitely non-specific in its action, since it inhibits the oxidation by tyrosinase irrespective of whether the substrate is p-cresol, l-tyrosine, l-dopa, adrenaline, or catechol-ascorbic acid (7).

No marked specificity among the test compounds studied was seen in the adrenaline-tyrosinase systems, benzoic acid, aniline, and the three isomeric aminobenzoic acids being effective in protecting adrenaline. This result is particularly noteworthy in view of the observations on the anti-sulfanilamide action of para-aminobenzoic acid and local anesthetics, such as benzocaine and novocaine derived therefrom, and the ineffectiveness as anti-sulfonamides of ortho and meta aminobenzoic acids, benzoic acid, and p-nitrobenzoic acid (8, 9).

Dopa occupies a unique position in the systems studied. It forms a pressor amine when incubated with minced kidney tissue, and yet it is capable of catalysing the oxidative destruction of adrenaline. Therefore, the behavior of a given compound at a given concentration in a tyrosinase system need not indicate its ultimate effect in the hypertensive animal. A molecule which inhibits tyrosinase and catalyses decarboxylase reactions will be materially different in its in vivo action from one which inhibits both tyrosinase and decarboxylase.

SUMMARY

1. The oxidative destruction of adrenaline, a typical pressor amine, is promoted in a tyrosinase-adrenaline system by ortho-substituted phenols and inhibited by aromatic amines and aminobenzoic acids.

2. The destruction of pressor bodies formed during incubation of dopa-kidney tissue systems is altered by the test compounds in the same manner as in tyrosinase-adrenaline systems.

3. The effectiveness of tyrosinase in reducing blood pressure of the perinephritic-hypertensive dog is enhanced by the simultaneous administration of catechol.

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STUDIES OF THE METABOLISM OF BOVINE EPIDIDYMAL SPERMATOZOA[1]

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The metabolism of mammalian spermatozoa has been studied previously. Attempts to identify the source of energy for motility and the development of methods for the assay of the fertilizing capacity of semen have been in the foreground of such investigations. The studies were based partly on chemical determinations of metabolic products, partly on manometric measurements of the gaseous exchange.

The present experiments were performed to confirm and extend observations made by several authors on the metabolism of bovine spermatozoa (1–4) which should serve as a basis for more extensive investigations of the effect of various reagents on the activity of these cells, particularly in relation to the enzyme systems present. Some initial studies of this nature have been completed (5, 6). Aspects of respiration and glycolysis of epididymal spermatozoa have been studied and factors responsible for variations in oxygen consumption have been analyzed. Results obtained with a few samples of seminal spermatozoa are included.

MATERIALS AND METHODS. *Spermatozoa.* Bovine testicles were obtained daily from the abattoir 1 to 2 hours after the death of the animals. Suspensions of spermatozoa were prepared in Ringer solution under conditions minimizing bacterial contamination as described elsewhere (7). Spermatozoa obtained from 6 to 10 epididymides were usually pooled. These heavy suspensions were diluted with Ringer solution so as to yield suitable concentrations for the tests. In a few instances the spermatozoa were washed once or thrice by slow centrifugation and subsequent resuspension in Ringer solution.

For a number of experiments bull semen was used.[2] A few of the samples were about 3 hours old; others had been kept at refrigerator temperature for 18 hours before use. The spermatozoa were sedimented in the centrifuge at low speed and resuspended in Ringer solution.

In the early work the spermatozoa were counted in a blood counting chamber after suitable dilution of the suspensions. In many instances the counting was

[1] This work has been aided by a grant from the National Committee on Maternal Health, Inc.

[2] Obtained through the courtesy of the Veterinary School of the University of Pennsylvania.

checked by determining the dry weight of the cells washed twice in distilled water and dried at 100°C for 18 hours. The average weight of 100 million bovine spermatozoa was found to be 2.4 mgm. More recently the counting has been replaced by measuring the turbidity of the spermatozoal suspensions by means of the Klett-Summerson photoelectric colorimeter. The number was estimated from a standard turbidity curve for suspensions of spermatozoa which were counted and diluted so as to yield concentrations of approximately 10^7, 2×10^7, and so forth, up to 10×10^7 cells per ml. The results obtained by counting and turbidimetry agreed within 10 per cent.

Media. Ringer solution containing 0.85 per cent NaCl, 0.2 per cent KCl, and 0.2 per cent $CaCl_2$ was used as basic medium. Usually glucose was added in a concentration of 100 to 200 mgm. per cent. In many cases M/15 phosphate buffers of different pH were added to the spermatozoal suspensions, ratios of 1:5 or 1:2 being used. For pH higher than 8.0 M/15 borate buffer was employed in the same ratios. For the determination of glycolysis sodium bicarbonate was added to the Ringer solution to a final concentration of 0.025 M.

Methods. The oxygen consumption of the spermatozoal suspensions was measured by means of the direct method of Warburg under air at 37°C. (8). Vessels with a central cup and one side-arm (volume 16 to 17 ml.) were employed. The CO_2 was absorbed by 0.3 ml. of 20 per cent KOH, which was placed in the central cup together with a piece of filter paper. The rim of the cup was covered with a thin layer of paraffin to prevent the alkali from creeping over into the spermatozoal suspensions. Two milliliters of the suspensions were always employed. Usually 2 vessels were used for each determination. The manometers were shaken at 110 oscillations per minute.

For the evaluation of the oxygen consumption a modification of the Warburg quotient

$$\left(Q_{O_2} = \frac{\text{cmm. } O_2}{\text{1 mgm. dry weight/hour}}\right)$$

was used (1b):

$$Z_{O_2} = \frac{\text{cmm. } O_2}{\text{100 million spermatozoa/hour}}.$$

This value may be converted into the more frequently used Q_{O_2} by dividing the Z_{O_2} values by 2.4, the dry weight of 100 million spermatozoa.

The respiratory quotient and aerobic glycolysis were determined in Dixon-Keilin vessels (8) under a mixture of oxygen and 5 per cent CO_2. The vessels were connected with Warburg manometers filled with Clerici solution. The calculations were made according to the second method of Dickens and Šimer (8).

In other cases the aerobic glycolysis was measured by the direct method of Warburg likewise under the O_2–CO_2 mixture. As the respiratory quotient of bull spermatozoa in the presence of glucose is 1.0 (5), this method can be substituted for the technique mentioned above. Comparison of the results obtained by both methods with the same spermatozoal suspensions showed negligible discrepancies.

The anaerobic glycolysis was determined by the direct method of Warburg in an atmosphere of nitrogen with 5 per cent CO_2. The glycolytic values are expressed as:

$$Z_G^{O_2} = \frac{\text{cmm. } CO_2 \text{ liberated by aerobic lactic acid formation}}{\text{100 million spermatozoa/hour}}$$

and

$$Z_G^{N_2} = \frac{\text{cmm. } CO_2 \text{ liberated by anaerobic lactic acid formation}}{\text{100 million spermatozoa/hour}}.$$

These values were calculated from the measurements for the first 30 minutes of an experiment.

Motility. The motility of the suspensions was checked at the end of each experiment and is described when pertinent to discussion of the results.

EXPERIMENTAL. *Oxygen consumption of bovine spermatozoa.* In confirmation of others (1, 2) bovine spermatozoa obtained from the epididymis were found to consume oxygen. Cells prepared as described and suspended in glucose-free Ringer solution showed a relatively high initial rate of oxygen consumption but it soon decreased (*A*, fig. 1) until it reached a rate (Z_{O_2}) of 3 to 4 cmm. oxygen for 100 million cells per hour. If glucose was added to the medium the oxygen uptake increased markedly and remained at the higher level (*B*, fig. 1), sometimes at a constant rate for 3 to 4 hours, when the experiments were terminated. Z_{O_2}-values as high as 25.0 were encountered under these conditions. Spermatozoa washed once or thrice and suspended in glucose-free Ringer solution did not show the initial high oxygen consumption of the unwashed cells (*C*, fig. 1.) and the Z_{O_2} for the first hour was already low and not higher than 5.0. This indicated that some substrate was transferred from the epididymis to the suspensions responsible for the higher respiration of unwashed cells in the first hour of the experiment but removed by washing of the cells. When glucose was added to washed spermatozoa the respiration increased to a level similar to that of the unwashed cells under the same conditions (*D*, fig. 1).

The motility apparently did not depend on the addition of glucose to the medium. At the end of the experiments washed spermatozoa in glucose-free medium exhibited motility comparable to cells suspended in Ringer glucose. Very dense suspensions of spermatozoa without added glucose showed motility even after 48 hours at room temperature.

It was found that as little as 1 mgm. per cent glucose when added to washed spermatozoa produced a short lived increase of respiration, the duration of which depended, of course, on the number of spermatozoa present. When 10 mgm. per cent of glucose were added to the medium, the respiration remained at the higher level for the length of the experiment, and further increase in the glucose concentration up to 300 mgm. per cent did not alter the Z_{O_2}-values. At no time in these experiments with epididymal spermatozoa did the addition of glucose cause a reduction in the oxygen consumed as described recently by Lardy and Phillips (4) for seminal spermatozoa.

The oxygen consumption of epididymal spermatozoa in the presence of glucose varied in the different experiments from 8.6 to 25.5 cmm. per 100 million cells. This variation called for further analysis and several factors were found to influence the oxygen uptake.

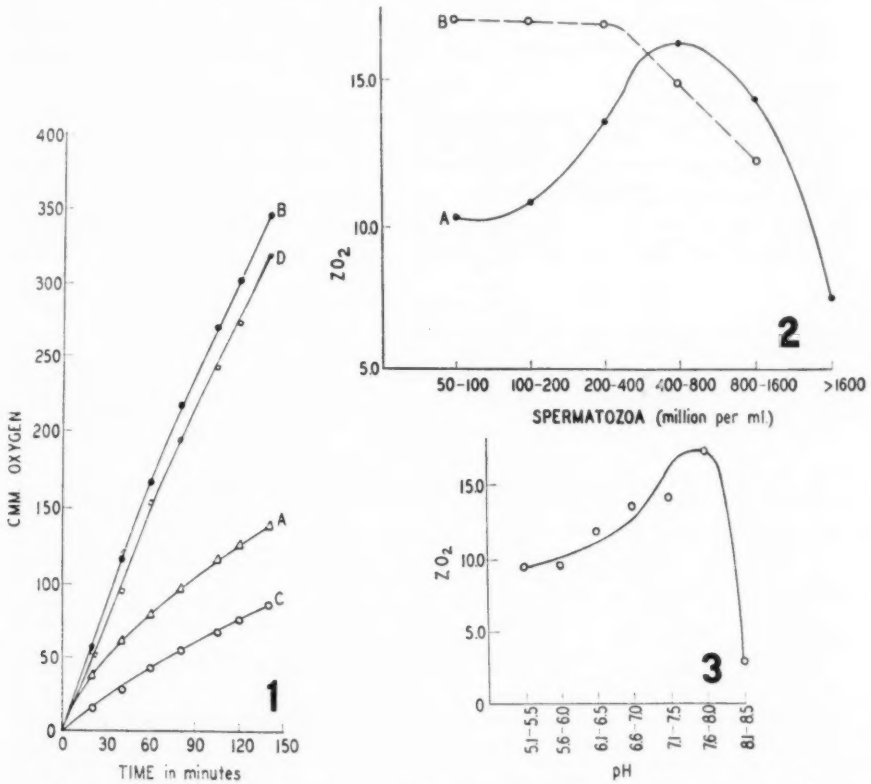

Fig. 1. Oxygen consumption of unwashed and washed epididymal spermatozoa (330 × 10^6 cells/ml) in Ringer solution with or without glucose. *A* = Unwashed spermatozoa in glucose-free Ringer. *B* = Unwashed spermatozoa in Ringer-glucose. *C* = Three times washed spermatozoa in glucose-free Ringer. *D* = Three times washed spermatozoa in Ringer-glucose.

Fig. 2. Influence of concentration of spermatozoa on oxygen consumption and the effect of epididymal secretion. *A* = Spermatozoa in Ringer-glucose. *B* = Spermatozoa in Ringer-glucose and epididymal secretion.

Fig. 3. Influence of pH on oxygen consumption of epididymal spermatozoa.

1. Differences in individual samples. Spermatozoal suspensions prepared from individual epididymides showed appreciable differences in respiration, as shown in table 1. The motility of the cells and the pH of the suspensions at the end of the experiments did not differ sufficiently to furnish an explanation for these variations.

2. Influence of the degree of maturity. Since spermatozoa undergo a process of maturation during their passage through the epididymis (9), attempts were made to obtain spermatozoa in various stages of development, i.e., from the testicle, the caput and the cauda of the epididymis, and from the vas deferens. However, it was found impossible to recover sufficient numbers of cells from some of the locations, and the spermatozoal suspensions were not free of blood and tissue cells. On the other hand, several samples of seminal spermatozoa,

TABLE 1

Oxygen consumption of bovine spermatozoa derived from individual epididymides, suspended in Ringer-glucose

SAMPLE NO.	NUMBER OF SPERMATOZOA PER ML.	Z_{O_2}			
		1st hour	2nd hour	3rd hour	Average
1	147×10^6	−10.7	−10.7	−10.8	
2	201×10^6	−20.6	−14.1	−14.4	
3	166×10^6	−13.3	−10.3	−10.4	−13.8
4	149×10^6	−12.5	−9.9	−10.8	
5	213×10^6	−22.6	−16.1	−16.2	
1–5	180×10^6	−15.2	−12.0	−12.1	−13.1

TABLE 2

Oxygen consumption of bovine seminal spermatozoa in Ringer-glucose

SAMPLE	NUMBER OF SPERMATOZOA PER ML.	AGE	MOTILITY	Z_{O_2}		
				1st hour	2nd hour	3rd hour
J	215×10^6	18 hrs.	+	−9.7	−6.9	−4.4
K	210×10^6	18 hrs.	+	−10.3	−5.6	−5.3
R	220×10^6	18 hrs.	—	0.0	0.0	0.0
10	500×10^6	3 hrs.	++	−6.9	−4.9	−3.5
9	450×10^6	3 hrs.	+	−7.6	−6.0	
epid. sp*	240×10^6	18 hrs.	++	−16.4	−15.7	−11.3
epid. sp†	345×10^6	18 hrs.	±	−7.0	−7.0	−7.2

* Whole epididymis kept in refrigerator.

† Spermatozoal suspension kept in refrigerator.

++ = very good motility; + = moderate motility; ± = majority immobile; − = no motility.

studied about 3 hours after emission showed an oxygen consumption decidedly lower than cells derived from the epididymis. Other samples, tested 18 to 20 hours after emission, until which time they had been kept in the refrigerator, consumed oxygen at a similar rate. Some of the data are recorded in table 2. One sample (R) which contained only immobile spermatozoa did not use oxygen. Suspensions of epididymal spermatozoa which were kept in the refrigerator for 18 hours showed similarly low values while storage of the whole epididymis did not affect the respiration of the spermatozoa subsequently prepared.

3. Influence of the concentration of spermatozoa on the oxygen uptake. It was

observed that higher Z_{O_2}-values frequently corresponded with a high spermatozoal count in the suspension. Experiments using various dilutions of the same suspension confirmed the observation. From 14 experiments an average curve has been computed (fig. 2, *A*) by plotting Z_{O_2}-values against the number of spermatozoa per milliliter. It is obvious from this figure that maximal respiration took place when the spermatozoal suspensions contained 400 to 800 million cells per milliliter. With higher concentration the oxygen uptake decreased. This fact could be due either to the effect of crowding with a resulting decrease of spermatozoal activity (cf. 10), or, particularly in the highest concentrations, to insufficient diffusion of oxygen into the suspensions. For the decrease in oxygen uptake encountered in dilute spermatozoal suspensions two possible explanations were considered: *a*, a factor in the epididymal secretion may stimulate the respiration if present in sufficient concentration, but is rendered ineffective by dilution although the actual amount of this substance per

TABLE 3

Influence of epididymal secretion on oxygen consumption of epididymal spermatozoa in Ringer-glucose (Z_{O_2})

NUMBER OF SPERMATOZOA PER ML.	CONTROL	EPIDIDYMAL SECRETION 10 MGM. PER ML.	PER CENT INCREASE
210×10^6	−9.8	−15.3	56
220×10^6	−8.4	−15.7	87
425×10^6	−10.3	−12.3	19
150×10^6	−9.4	−16.6	77
220×10^6	−13.7	−19.1	39
205×10^6	−10.0	−15.5	55
220×10^6	−9.8	−9.5*	

* Seminal fluid added instead of epididymal secretion.

spermatozoön would remain constant; *b*, products of spermatozoal metabolism may enhance the oxygen uptake in more concentrated suspensions, while they may not reach an effective level in dilute preparations.

In order to test these points the respiration of spermatozoal suspensions was studied in Ringer-glucose with or without the addition of "epididymal secretion". This material was obtained by collecting large amounts of spermatozoa in distilled water and separating the cells by centrifugation at 2000 r.p.m. for 30 minutes. The opalescent supernate was saved and dried from the frozen state. The reddish-brown brittle material was added to the spermatozoal suspensions by weight so as to yield a 1 per cent solution. Table 3 shows that this material stimulated appreciably the oxygen uptake of spermatozoa. The increase in respiration varied in some 20 experiments from 19 to 87 per cent over the controls, the average being 40 per cent. The epididymal secretion alone did not absorb oxygen or, if so, only minimal amounts, and the results of the experiments were corrected accordingly. The motility of the spermatozoa was not influenced by the agent.

As little as 2 mgm. of the material per milliliter gave the stimulating effect. Freshly obtained epididymal secretion gave a stronger effect than the dried material obtained from spermatozoal suspensions collected over several days. This indicated its epididymal origin rather than its derivation from spermatozoal metabolism or autolysis. The factor was found to be heat stable, withstanding 30 minutes at 56°C. or even 10 minutes at 100°C. without loss of activity. It was not dialysable through cellophane, i.e., the residue stimulated the respiration to approximately the same degree as the original material and the concentrated dialysate was without effect. The addition of seminal fluid to epididymal spermatozoa did not influence the oxygen uptake.

The increase in oxygen consumption induced by the addition of the epididymal secretion clearly depended on the concentration of spermatozoa in that the higher increases were observed in dilute suspensions, while in preparations containing more than 500 million cells per milliliter no stimulation was noticed or even a slight depression took place. Curve *B* of figure 2 represents the average of 4 such experiments. It shows that the drop in respiration encountered in dilute suspensions of the control spermatozoa can be prevented completely by the addition of the epididymal secretion.

4. Influence of pH. Epididymal spermatozoa suspended in Ringer solution showed a pH of 6.0 or slightly less. This agreed with the pH of the cauda of the epididymis which was found by Redenz (9) to be approximately 5.8. The pH of bull semen has been found to be 6.5 to 7.5 (11). When epididymal spermatozoa were suspended in Ringer-glucose solution buffered at various pH the respiration showed an optimum between pH 7.5 and 8.0 both in phosphate and borate buffers, and so did the motility. Above this value the oxygen uptake dropped rapidly while below the optimal pH the respiration fell more gradually until a pH of 5.0 was reached, the lowest pH studied with the phosphate buffer. These relationships are demonstrated in figure 3, which represents an average curve drawn from eight different experiments covering this range of pH.

5. Influence of various media. It is reasonable to assume that media of various composition will influence the oxygen uptake of bovine spermatozoa. However, this point has not been studied adequately and only experiments comparing the respiration in Ringer-glucose and in Ringer-bicarbonate-glucose have been made. The oxygen uptake was markedly increased in the latter medium and Z_{O_2}-values of over 30 were encountered. The motility also seemed more active in this medium than in the bicarbonate-free preparations.

Aerobic and anaerobic glycolysis. Manometric data concerning the aerobic and anaerobic glycolysis of bovine spermatozoa are available through the studies of Redenz (1), who used dialyzed serum as medium. In the present study the spermatozoa were suspended in Ringer-bicarbonate solution to which 200 mgm. per cent of glucose had been added. Table 4 shows some of the results which agree well with the observations of Redenz. The values for aerobic and anaerobic glycolysis varied to a certain degree in different samples but less than the oxygen consumption. The concentration of spermatozoa per milliliter did not play a rôle comparable to that in the respiration experiments nor did the epididymal secretion influence the lactic acid formation.

Since only very few samples of bovine seminal spermatozoa were available for the determination of glycolysis, a comparison between these and epididymal cells in regard to glycolysis does not seem justified, although in the few tests performed the rate of anaerobic glycolysis appeared somewhat smaller in the seminal spermatozoa. MacLeod (12) observed that human seminal spermatozoa showed an aerobic glycolysis amounting to 80 per cent of the anaerobic one. In the present experiments with bovine epididymal spermatozoa the aerobic glycolysis was found to be 40 to 60 per cent of the anaerobic values. Two samples of seminal spermatozoa showed an aerobic glycolysis of 60 and 73 per cent of the anaerobic lactic acid formation.

Discussion. In accordance with Redenz (1) it was found that the oxygen consumption of bovine epididymal spermatozoa varied over a wide range. In analyzing this phenomenon it was observed that spermatozoa derived from individual epididymides showed different oxygen needs. Spermatozoa undergo a process of maturation while passing through the epididymis (9). On frequent emission the cells become more and more immature, while on the other hand

TABLE 4

Aerobic and anaerobic glycolysis of bovine spermatozoa

SOURCE OF SPERMATOZOA	NUMBER OF SPERMATOZOA PER ML.	$Z_G^{O_2}$	$Z_G^{N_2}$	$Z_G^{O_2}$ AS PER CENT OF $Z_G^{N_2}$
Epididymis	910×10^6	56.7	95.4	59.4
Epididymis	470×10^6	37.5	95.7	39.2
Epididymis	340×10^6	37.7	73.0	51.6
Epididymis	115×10^6	38.0	84.0	45.3
Epididymis	130×10^6	48.6	81.2	60.0
Epididymis	157×10^6	38.2	76.3	50.0
Semen	500×10^6	44.0	60.0	73.3
Semen	450×10^6	44.5	73.4	60.6

spermatozoa stored for a long period in the epididymis lose gradually their fertilizing capacity (cf. 13). These physiological changes may account for some of the individual differences found in this study in that spermatozoa of varying degrees of maturity may have different metabolic requirements. Although the various developmental stages could not be tested, results obtained with seminal and epididymal spermatozoa showed that the oxygen uptake was so much lower in the former that there is no doubt that respiration is less in seminal cells. This difference has to be studied further. Possible interaction of the secretions of the accessory glands may be excluded since the addition of seminal fluid to epididymal cells did not alter their oxygen consumption. Lardy and Phillips (4) found that the oxygen consumption of seminal cells is lower in the presence of glucose than in glucose-free medium, a fact we can confirm for seminal spermatozoa but not for epididymal cells.

Optimal oxygen consumption took place in suspensions containing 400 to 800 million cells per milliliter. The drop in respiration encountered in excessively concentrated suspensions may be ascribed to insufficient diffusion of oxygen into the medium or may partly be due to a crowding effect (cf. 10). The decreased

oxygen uptake in dilute suspensions of spermatozoa could be overcome by the addition of epididymal secretion to the medium. The agent present was found to be heat stable and not dialysable. Seminal fluid did not influence respiration.

Finally the pH may alter the oxygen uptake, optimal respiration taking place at pH 7.5 to 8.0. By controlling the various factors mentioned it has been found possible to obtain more uniform results and the variation in oxygen consumption observed under these conditions was not greater than encountered in other mammalian cells and tissues.

In agreement with others (3, 4, 12 and others), a high aerobic glycolysis was found in addition to the anaerobic glycolysis. There is no doubt that the glycolysis furnishes the major part of energy for spermatozoal activity in the presence of glucose. However, respiration and other processes may substitute under certain conditions (cf. 14).

SUMMARY

Variations in the oxygen uptake of bovine epididymal spermatozoa, suspended in Ringer solution, were shown to be due *a*, to differences in the individual samples; *b*, to the concentration of spermatozoa, optimal respiration taking place in suspensions containing 400 to 800 million cells per ml.; *c*, to the pH of the suspensions, highest oxygen consumption occurring at pH 7.5 to 8.0; *d*, to possible differences in the degree of maturity of the cells. Seminal spermatozoa showed a decidedly lower oxygen consumption than epididymal cells.

An analysis of the phenomenon of optimal concentration showed that while in excessively concentrated suspensions mechanical hindrance of the spermatozoa or insufficient diffusion of oxygen into the medium could account for the lower oxygen uptake, the decreased respiration in dilute suspensions of spermatozoa was completely prevented by the addition of epididymal secretion to the medium. The factor present in this material is heat stable and not dialysable.

The high aerobic glycolysis of epididymal cells, amounting to 40 to 60 per cent of the anaerobic reaction, was confirmed. The concentration of the spermatozoa did not influence the amount of glycolysis and epididymal secretion did not alter the results.

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THE USE OF DOUBLE WORK PERIODS IN THE STUDY OF FATIGUE AND THE INFLUENCE OF CAFFEINE ON RECOVERY[1]

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A review of the literature reveals that a "single work period" performed one or more times per week is the method most commonly employed for the study of the effect of various factors on voluntary motor fatigue (1–10). The published observations show that training, "staleness" and other known and unknown factors cause a variation in the output of work from period to period. The factors of training, "staleness," motivation or the "will to do," are factors difficult to control. Our work was undertaken to ascertain whether double work periods, in which the subject works to exhaustion, then rests for a brief period and then again works to exhaustion, might be more advantageous for the study of fatigue than the "single work period." It was believed that the per cent recovery might be more constant than the output of work during a "single work period." Certain observations reported by a number of investigators indicated that such might be the case (11–17). In fact, one investigator (17) has studied the effect of massage, etc., on the per cent of recovery.

This paper presents the results obtained regarding whether the per cent of recovery and other items of data provided by double work periods is more constant than the output of work during a single work period. Results on the effect of caffeine on the per cent of recovery and the output of work during a second work period after a ten minute period of rest are also presented.

Caffeine was chosen as the drug to use in these tests because it is generally recognized to be a drug which increases the work output in voluntary muscular fatigue in egometric tests (18, 19) as usually performed. However, the drug has not been administered intravenously in man to ascertain its effect on recovery from fatigue or rather its effect on voluntary muscular effort in the presence of fatigue. The nearest approach was made by Graf (14, loc. cit. Bøje) who administered caffeine and cola nuts after partially fatiguing his subjects.

METHODS. Six medical students were used. They were provided with board and room in a hospital near the laboratory. Their food intake was under control; their diet was adequate in all known components, a certain variation in the selection of food items being permitted. The subjects were chosen on the basis of their willingness to coöperate, and not on the basis of muscular development, the work of the tests being their only source of physical exercise. The

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double work periods occurred on Monday, Wednesday and Friday afternoons at the same time between 2 and 4 o'clock.

The subjects worked on the bicycle ergometer described by Hellebrandt and Kelso (20). After trying various rates, loads and rest periods on a relatively large number of subjects, a rate of work of 1235 kg. m. per minute with a pedalling rate of 54 r.p.m. was chosen for the tests. Three to five preliminary workouts on the bicycle were allowed for the development of a skill pattern.

The subjects worked to complete fatigue, rested ten minutes, and then worked to complete fatigue again. The ten minute rest period was chosen because some subjects if given longer periods of rest manifested complete recovery. Indications that the subjects had gone the limit each time were profuse perspiration, uncontrollable muscular twitching, spasm and contracture of the quadriceps, leg

TABLE 1

Comparison of the variability of the first period, second period, and total work output as well as that of per cent recovery

SUBJECT	PERIOD OF TRAINING	NUMBER OF DOUBLE WORK PERIODS	AVERAGES WITH STANDARD DEVIATION				COEFFICIENT OF VARIATION (PER CENT)			
			1st period work output	2nd period work output	Total work output	Per cent recovery	1st period work output	2nd period work output	Total work output	Per cent recovery
	months		*Kg. m.*	*Kg. m.*	*Kg. m.*					
A	2 -3	19	13,951 ∓1913	6356 ∓ 677	20,275 ∓2529	46 ∓5.5	13.72	10.65	12.48	11.95
B	1.5-3.75	21	6,573 ∓ 813	4491 ∓1447	11,142 ∓1197	68 ∓5.67	12.23	32.21	10.76	8.34
C	2.5-3.75	19	10,378 ∓1859	6125 ∓ 744	16,510 ∓2213	60 ∓9.11	17.93	12.14	13.41	15.20
D	2 -3.25	15	10,029 ∓ 803	5595 ∓ 417	15,540 ∓ 962	56 ∓5.70	8.02	7.45	6.21	10.17
Average of A, B, C, D						57 ∓6.5	13.22	15.59	10.71	11.41
E	0 -1.5	17	2,592 ∓ 426	2107 ∓ 276	4,700 ∓ 686	81 ∓5.55	16.42	13.08	14.59	6.80
F	0 -1.5	12	2,408 ∓ 359	1946 ∓ 280	4,355 ∓ 630	81 ∓3.41	14.92	14.38	14.45	4.20
Average of E and F							15.67	13.73	14.52	5.50
Average of all						∓5.82	13.87	14.99	11.98	9.44

buckling, leg pains and slight nausea. The end-point of fatigue was the point at which they could no longer hold the needle on the dial at the rate of 54 r.p.m.

RESULTS. A summary of a portion of the data on the work output of the six subjects is shown in table 1. It is to be noted that in the case of the first four subjects no data are given for the first 1.5 to 2.5 months. This is because these subjects were used during that period to survey various factors that might influence the duration of the first and second work periods, such as the load, pedalling rate, etc., factors that we desired to study but which do not pertain to the subject of this paper. Hence, the data shown for the first four subjects represent those obtained after a brief period of training. The data on subjects E and F, however, represent the initial period of training. It is to be noted that the average standard deviation of the *per cent recovery* for each subject is relatively small and that the average standard deviation of the group is only 5.82

per cent. The coefficient of variation (21) of the per cent of recovery is less than that for the first work period in all cases, except subject D, and less than that for the second work period in three of the six subjects. In the case of subjects E and F, who started untrained with the standard rate of work, the data represent their process of early training. Both have shown substantial increases in work output with training. In the case of subject E the work output during the first period was 2.4 times more variable than his per cent of recovery; in the case of subject F, 3.5 times more variable.

Comment. In the first place the double work period, as used, provides four items of data which may be studied statistically instead of the one item provided by the single work period. Secondly, it is evident that in five of the six subjects

TABLE 2

SUBJECT	PERIOD OF TRAINING	NUMBER OF DOUBLE WORK PERIODS	AVERAGES WITH STANDARD DEVIATION OF THE DISTRIBUTION				COEFFICIENT OF VARIATION (PER CENT)			
			1st period work output	2nd period work output	Total work output	Per cent recovery	1st period work output	2nd period work output	Total work output	Per cent recovery
			A. With intravenous placebo of NaCl solution							
	months		*Kg. m.*	*Kg. m.*	*Kg. m.*					
A	4 -6	11	9,948 ∓1,822	5880 ∓ 358	15,830 ∓1972	60.7 ∓ 9.52	18.30	9.36	12.46	15.68
B	4 -6	10	6,560 ∓ 249	4722 ∓ 214	11,280 ∓ 427	71.9 ∓ 3.15	3.80	4.53	3.78	4.38
E	1.5 -3.25	10	3,836 ∓ 289	2777 ∓ 157	6,614 ∓ 426	72.7 ∓ 3.29	7.54	5.66	6.44	4.52
F	1.25-3	10	3,993 ∓ 480	2594 ∓ 256	6,522 ∓ 717	65.3 ∓ 4.45	15.27	9.88	10.99	6.81
Averages						67.49 ∓ 5.85	11.23	7.36	8.42	7.85
			B. With intravenous caffeine-sodium benzoate 0.5 gram							
A	4 -6	11	10,043 ∓ 1313	9006 ∓1167	20,891 ∓2231	91.5 ∓18.64	13.06	12.96	10.68	20.37
B	4 -6	10	6,481 ∓ 294	5559 ∓ 285	12,039 ∓ 481	86 ∓ 4.90	4.54	5.13	4.00	5.70
E	1.5 -3.25	10	3,606 ∓ 380	2836 ∓ 112	6,442 ∓ 483	79.2 ∓ 5.74	10.54	3.95	7.50	7.25
F	1.25-3	10	3,641 ∓ 467	2595 ∓ 348	6,236 ∓ 789	71.4 ∓ 4.63	12.83	13.41	12.65	6.48
Averages						82.2 ∓10.6	10.24	8.86	8.76	9.95

* One gram of caffeine sodium benzoate was required to produce the same effects in subjects E and F as were produced in subjects A and B with the half-gram dose.

the per cent recovery showed less variation than the output of work during the first work period In the exceptional case, subject D, the total work output as well as that during the first or second work periods is less variable than the per cent of recovery. In the case of subjects E and F, it may be concluded that any test factor or drug, introduced at random during the period of training, which increases the per cent of recovery two or more times the standard deviation above the mean of the placebo tests, has a favorable effect on recovery. Thus, the effect of drugs and other quickly acting substances on the fatigue mechanisms can be assayed during the training period, and the results would be as reliable as results obtained after a period of training. Referring to table 2A, it will be noted that the coefficient of variation of the per cent recovery of the same subjects (E and F) is significantly no more variable during the first portion of the

period of training than later. The same is only relatively true of subject A, but subject B has become more consistent with further training.

Comparison of the variation of the work outputs of the first work periods of our subjects with those of others. Doctor Hellebrandt provided us with data on six control subjects who started training on the bicycle ergometer under standard conditions, as did our subjects E and F. She used only a "single work period." The average coefficient of variation during the first 15 work periods (first 5 omitted to allow for development of a skill pattern as was done in our tests) in her subjects was found to be 14.05 per cent, and that of our two comparable subjects, E and F, during the first work period was 15.67 per cent, which is remarkably close.

Four of her subjects continued the tests for two months and the other two for six weeks longer. For the tests performed after the initial training period the average coefficient of variation of the four subjects stopping at two months was 9.17 per cent, and for the two stopping at 3.5 months was 5.0 per cent. The average coefficient of variation of the per cent of recovery in subjects E and F during the first six weeks of training was 5.5 per cent. This indicates that per cent recovery, as a criterion of fatigue, is in some subjects as constant during the first six weeks of training with subjects working only 3 days a week as is the single daily workout after a period of two months of training in some subjects. Refering to table 2A, which summarizes our data on four subjects during a period of from 1.5 to 6 months, it is seen that the coefficient of variation during the first work period in the different subjects varies from 3.8 to 18.3 per cent, giving an average of 11.23 per cent Two of our subjects, B and E, fell within the range of the two of Doctor Hellebrandt's subjects who worked for 3.5 months. But it should be noted that in 3 of our 4 subjects (table 2A) the per cent of recovery was less variable than the work output of the first work period.

Further evidence of the constancy of per cent recovery was obtained by interpreting Kent's (13) data statistically. He gives 60 consecutive per cent recoveries on one subject. Thirty were taken in the morning and 30 in the evening. The average morning recovery was 90 $\pm$ 9.51 per cent, giving a coefficient of variation of 10.56 per cent. The average evening recovery was 88.5 $\pm$ 9.08 per cent, giving a coefficient of variation of 10.26 per cent. The initial and second work outputs were not given. Considering the fact that Kent's data were collected using the finger ergograph with a 5 minute rest period, it is remarkable that the average coefficient of variation of 10.41 per cent, calculated from his data, so closely approximates our average coefficient of variation of 9.44 per cent.

In regard to the use of the *finger ergograph*, we initially started to use the extensor ergograph, modified after Maison (22), but were dissatisfied with the variation observed from day to day. In our subject the average 1st period work output for 33 tests was 11.7 $\pm$ 4.89 kgm. giving a coefficient of variation of 43.78 per cent. Doctor Maison kindly permitted us to analyze the data he had obtained on himself; the coefficient of variation of the first twenty-five work periods was 47.51 per cent. Even using the extensor ergograph with a 1 minute rest period, we found the per cent recovery in our one subject was 40 $\pm$ 11.6 per

cent, giving a coefficient of variation of 29 per cent. This is supported by interpreting Lamb's data (25) statistically. He used an arm ergograph and fatigued the right arm in 28 subjects and the left in 25. The subjects were untrained and each served for one test. The arm was worked to fatigue, rested 10 minutes, and worked to fatigue again. The average coefficient of variation for the 1st work period (both arms) was 41.25 per cent; that for the per cent recovery was 31.01 per cent. This indicates that *even in untrained individuals per cent recovery is a more constant physiological character than is the initial work output.*

Individual differences. The individual differences in the work output and per cent of recovery during the earlier and later portions of the training or experimental period should be noted in tables 1 and 2. In the case of subject A, the per cent recovery increased after three months; however, the total work and especially the work output during the first period decreased. Subject B has manifested little change in recovery and work output after 3 months; he is a very consistent subject. Subjects C and D discontinued the experiment after 3.5 months. Subjects E and F manifested a decrease in per cent recovery after 1.5 months, but the total work output and especially that of the first period increased.

The averaged data (tables 1 and 2) as well as the data of the individual tests show that the per cent of recovery is inversely related to the work output of the first period, as might be anticipated, even though the former is less variable; the correlation coefficient is 0.95. As the subject trains and becomes capable of a greater output during the first period, the per cent recovery becomes less. This indicates that a given subject recovers from fatigue more rapidly when he works to "exhaustion" during the early period of training, when the output of work is relatively small, than he does later when the output of work is greater. As training progresses, the load becomes relatively lighter for the muscles and the factors concerned in fatigue may accumulate less acutely; this permits the accumulation of a greater debt, which requires a longer recuperative period. As training progressed, the subjects reported that pain in the muscles became less significant as a cause of cessation of work. Another point of interest is that with additional training the output of work during the second period became less variable (tables 1 and 2) and on the average (table 2) was slightly less variable than the per cent of recovery. With training the coefficient of variation of the per cent of recovery may increase or decrease; it increased in subjects A and F, and decreased in subjects B and E (tables 1 and 2).

The Effect of Caffeine. Methods. Four subjects were used. Two (A and B) had been trained for three months, and two (E and F) for about 1.5 months. Immediately after the first work period, the subjects were injected at random intravenously with a placebo (2 cc. NaCl solution) and 0.5 gram of caffeine-sodium benzoate (2 cc.) or 0.25 gram caffeine. Ten minutes after the end of the first work period, the subjects worked to exhaustion again. The two solutions were colorless. Coffee had not been taken by any of the subjects for several months, and was not taken during the experiment. Subject A weighed 155 lbs.; B, 155 lbs.; E, 140 lbs.; and F, 180 lbs.

Results. The summarized data are shown in tables 2A and 2B. The average

per cent recovery for the four subjects was 67.49 ± 5.85 with the placebo and 82.2 ± 10.6 with the caffeine. The effect of caffeine was definite; the critical ratio of the difference is 7.7. The critical ratio for the difference between the placebo and caffeine tests in the case of subject A is 4.4, and subject B, 4.8; for the other two subjects, it is 2.7. Referring to the *work output during the second period*, the critical ratio for the difference between the placebo and caffeine tests for subject A is 7.6; for subject B, 6.6; for subjects E and F there is no difference. Referring to the *total work output*, the critical ratio for the difference between the placebo and caffeine tests for subject A is 5.1; for subject B, 3.3; and for subjects E and F there is no significant difference.

Comment. In the case of subjects A and B, the critical ratios (greater than 3) of the differences between the placebo and caffeine tests are significant for per cent recovery, for work output during the second period or the period after caffeine, and also for the total work output for both periods. The caffeine, thus, had a definite effect on subjects A and B. Although the critical ratio for the difference between the placebo and caffeine tests relative to the per cent of recovery comes close to being significant, the lack of a difference between the output of work during the second period indicates that the difference in per cent recovery is probably due to chance, in the case of subjects E and F.

The reports of the influence of caffeine on the feelings of the different subjects correlate with the objective results just cited.

Subjectively A and B reported that when they received the caffeine, though they did not know what it was, they felt less fatigued during the rest period and that they believed the onset of fatigue and muscular pain during the second work period was delayed. Subject E was unable to tell whether he received caffeine or a placebo and F had a bitter taste after the caffeine, but had no other sensations. Apparently the dose of caffeine used was not sufficient to influence subjects E and F. Hence, the increased output of work by subjects A and B is best ascribed to the elevation in mood and the decrease in the sensation of fatigue produced by caffeine. However, it should not be forgotten that subjects A and B were doing more work than subjects E and F. In subjects E and F a one gram dose of the drug was required to produce objective and subjective effects similar to those obtained by a half gram dose in subjects A and B.

Graphic records of *hand tremor* showed that caffeine increased the tremor slightly; the records were made directly after the end of the second work period. The caffeine had no effect on *breath holding* tests made 10 minutes after the end of the second work period.

SUMMARY AND CONCLUSIONS

Six subjects under controlled conditions of housing and diet were studied to ascertain whether a "double work period" might be more advantageous for the study of fatigue than the "single work period." A bicycle ergometer and a rather heavy load was used, 1235 kg. m. per minute with a pedalling rate of 54 r.p.m. Three times each week the subjects were worked to exhaustion, and after ten minutes of rest were worked to exhaustion again. In three of four subjects,

A, B and C, who had been previously trained for a period of from 1.5 to 3.75 months, the per cent of recovery was found to be less variable than the work output of the first or second periods. The total work output for the two periods (subjects A, B, C, and D) varied to about the same extent as the per cent of recovery. In two subjects, E and F, who had not been previously trained, the per cent of recovery was less variable during 1.5 months of training than any other item of the data; the total work output for the two periods ranked second. Further, training of these two subjects for a total period of 3 months showed that the per cent of recovery continued to be the least variable. Further, training of subjects A and B for a total period of 6 months showed the work output during the second period to be the least variable. *In general, as the length of the training period increases the work output during the second period, the total work output for both periods and the per cent recovery become less variable and approximately equal in variability; the work output during the first period remains the most variable of the four items of data.* The double work periods in three of four of our subjects even after considerable training yielded three items of data less variable than the single or first work period (table 2).

Another advantage of the double work periods is that it permits a drug or rapidly acting substance to be assayed during the training period, so that each subject may serve as his own control. In addition, it permits a study of the effect of rapidly acting substances on recovery.

It was found, as might be anticipated, that the per cent recovery under our experimental conditions is inversely related to the work output of the first period; the correlation coefficient was approximately 0.95.

Caffeine-sodium benzoate (0.5 gram) injected intravenously at the end of the first work period increased the per cent recovery or the output of work during the second period in the two subjects that were subjectively affected by the drug. It had no significant effect in the two subjects who were not subjectively affected; in these subjects a one gram dose of the drug was required to produce objective and subjective effects.

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THE CIRCULATORY RESPONSE OF THE UNANESTHETIZED DOG TO SMALL PHYSIOLOGICAL QUANTITIES OF ADRENALIN

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There is general agreement that the injection of small quantities of adrenalin produces marked changes in arterial blood pressure and readjustments in blood distribution (1, 2 and others). Several factors may participate in determining the direction and magnitude of this response (3). For example, the low blood pressure which follows pithing may be elevated by the injection of adrenalin in amounts which prior to pithing elicit a depressor response (4). A similar reversal of the adrenalin depressor response occurs when anesthesia deepens (5). Minimal effective doses of this drug are said to reduce the arterial blood pressure in the etherized dog (6) and in the decerebrate cat (7). Such findings suggest that adrenalin depressor responses should be readily elicited in normal, unanesthethized animals. Dragstedt and his associates (8), however, encountered only pressor responses to adrenalin injections in unanesthetized dogs. They postulated in addition that the minimal effective dose of adrenalin for these animals ranges from 0.2 to 0.4 μg./kgm./min. In the present study an attempt has been made to investigate the circulatory effects of small physiological doses of adrenalin in the unanesthetized dog with the aid of an optical manometer system.

METHODS. Seven dogs which were accustomed to the technical procedure served as the experimental animals. In all experiments the dogs were unanesthetized and had been deprived of food for at least sixteen hours. The animals rested quietly for thirty to forty-five minutes before any measurements of the blood pressure were made. The marked phasic sinus arrhythmia, normally present in quiet dogs, was occasionally reduced or eliminated by the onset of panting induced by high environmental temperature and humidity. It is worthy of mention, however, that the hemodynamic changes in such instances were identical both in direction and magnitude with those observed during quiet breathing.

Systolic and diastolic pressures were recorded from the femoral arteries by means of a modified Gregg hypodermic optical manometer system (9). Hypodermic puncture of the jugular vein was also carried out for the administration of adrenalin. To eliminate any disturbances associated with the act of puncturing arteries and veins, 30 to 60 seconds were allowed to elapse before actual registration of blood pressures was begun.

When arterial pressures are determined by this technique, the slightest blood

clotting within the lumen of the needle may produce bizarre pulse contours and, consequently, unreliable ordinate pressure values. Systemic anti-coagulants cannot be employed because the procedure requires repeated arterial punctures. The adrenalin response has therefore been studied for short intervals only following single injections of the drug. The blood pressure was recorded upon rapidly moving photosensitive paper. This procedure permitted careful study of the individual pulse curves by means of which coagulation within the system was detected and the faulty records discarded.

As a precautionary measure against deterioration of the adrenalin, fresh, concentrated stock solutions (1:1000) were diluted to the desired strength immediately prior to administration. Distilled water and physiological saline served as alternate diluents. The arbitrarily selected quantities of adrenalin (0.1, 0.2 and 0.3 μg/kgm.) were prepared in volumes which never exceeded 3 ml. of fluid and were administered via the jugular veins. External compression of the veins was carefully avoided. The rate of injection was varied from 0.019 to 0.1 μg./kgm./sec.

Results. A special procedure for record analysis was adopted in order to discount the large fluctuations in blood pressure determinations which are primarily respiratory in origin. After the systolic and diastolic values for every individual pulse had been determined, each record was arbitrarily divided into six-second pre- and post-injection intervals, commencing with the onset of injection. An average of the pressures for each interval was obtained and the respective heart rates counted. The average blood pressures from interval to interval in the control periods were very constant. In 28 observations of control blood pressures, the systolic and diastolic pressures averaged 190 and 90 mm. Hg respectively. The latter compare favorably with normal femoral pressure values similarly procured by Hamilton (10) in 215 unanesthetized dogs. An average heart rate of 97 beats per minute was obtained from all control records. If, however, the records registered on panting dogs are excluded, the latter value is lowered to 87 beats per minute. Blood pressure levels were not measurably affected by the onset of panting.

The necessity of insuring adequate separation of the individual pulse curves entailed photographic registration of exceptionally long records. Since even the inclusion of typical segments of the latter was found impractical, the experimental observations are presented in tabular and graphic form. The direction and magnitude of the maximal circulatory responses from individual experiments are entered in table 1. Associated with the latter are *a*, the dose of adrenalin employed, *b*, the rate of injection, and *c*, the total volume of fluid administered. In these studies it was impossible to detect any significant correlation between the rate of injection and either the direction or magnitude of the adrenalin depressor response. A similar lack of correlation has been more recently observed in experiments in which large physiological pressor doses were administered at rates as great as 0.1 μg./kgm./sec. (11). Control injections of the diluents in volumes equivalent to the maximal quantities required for adrenalin administration altered neither the heart rate nor the blood pressure.

There is evidence, however, that the adrenalin concentration per unit of animal weight determines the direction of the circulatory response. In 13 of 14 tests, for instance, arterial pressures were definitely lowered following injection of 0.1 μg./kgm. quantities of this drug. As the strength of adrenalin was

TABLE 1

DOG NO.	EXP. NO.	DOSE	DOSE	MAX. CHANGE IN S.P.	MAX. CHANGE IN D.P.	MAX. CHANGE IN H.R.	ADRENALIN INJECTED	NATURE OF RESPONSE
		μg./kgm.	*μg./kgm./second*				*ml.*	
1	19	0.1	0.071	−28	−18	+25	0.78	Depressor
	20	0.1	0.071	−26	−11	+14	0.78	Depressor
	22	0.2	0.100	+4	+6	+5	1.76	
	21	0.3	0.100	+11	+13	−9	2.34	
	25	0.3	0.050	−22	−9	+42	2.43	Depressor
2	13	0.1	0.019	−26	−15	+20	0.86	Depressor
	15	0.1	0.037	−23	−22	+44	0.86	Depressor
	16	0.2	0.044	+26	+15	−25	1.70	
	17	0.3	0.050	+55	+29	−40	2.60	
	20	0.3	0.070	+25	+17	−22	2.65	
3	17	0.1	0.009	−25	−11	+30	0.60	Depressor
	20	0.1	0.009	−11	−14	+26	0.60	Depressor
	21	0.2	0.040	−34	−10	+45	1.16	Depressor
4	12	0.1	0.044	+30	+2	+15	0.66	
	13	0.1	0.026	−16	−5	+13	0.66	Depressor
	30	0.1	0.080	−22	−2	+38	0.66	Depressor
	14	0.2	0.080	−21	−2	+52	1.32	Depressor
	15	0.3	0.070	−20	−5	+43	1.98	Depressor
5	19	0.1	0.034	−34	−26	+34	1.18	Depressor
	20	0.1	0.040	−32	−17	+36	1.18	Depressor
	21	0.2	0.040	−50	−22	+27	2.36	Depressor
6	1	0.1	0.039	−27	−8	+40	0.94	Depressor
	5	0.1	0.027	−11	−3	+30	0.99	Depressor
	11	0.2	0.083	+18	+11	+40	1.98	
	2	0.3	0.071	−26	−9	+36	2.82	Depressor
7	7	0.1	0.040	−12	−4	+14	1.13	Depressor
	12	0.3	0.088	+17	+10	−15	3.15	
	5	0.3	0.100	−16	−15	+33	3.42	Depressor

increased, pressor elevations were also observed and the number of depressor responses obtained became less frequent. For example, the depressor response was encountered in only 66 and 63 per cent of the experiments in which 0.2 and 0.3 μg./kgm. doses were respectively introduced. With 0.5 μg./kgm. dosages, 18 of 20 trials revealed pressor reactions. Excluding the 0.1 μgm. doses, all

other quantities of adrenalin were occasionally observed to elicit dual responses, characterized by an initial depression and a secondary rise in the blood pressure.

It is difficult to detect the factor or factors which govern the magnitude of this depressor response, particularly since each of the specific doses included in table 1 has, at one time or another, produced both great and small reductions in arterial pressure. The largest reductions in systolic and diastolic pressures encountered were respectively 50 and 26 mm. Hg and the smallest 11 and 2 mm. Hg. The average systolic and diastolic reductions were 25 and 11 mm. Hg.

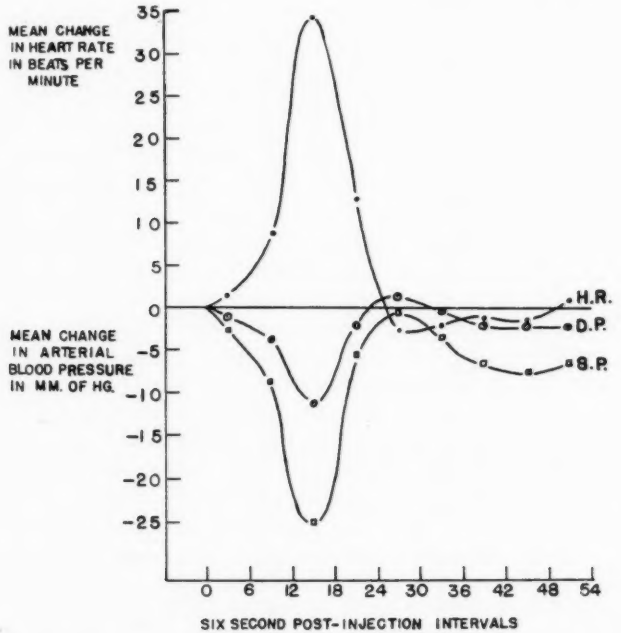

Fig. 1. Mean duration-response curves compiled from 28 experiments in which a depressor response was obtained with 0.1, 0.2 and 0.3 μg./kgm. doses of adrenalin. *H.R.*, heart rate; *D.P.*, diastolic pressure; *S.P.*, systolic pressure.

Since the respiratory fluctuations have been previously discounted in the calculations, these changes are significant.

Inasmuch as the *directional* changes in heart rate and blood pressure were so strikingly uniform during the respective post-injection intervals of each experiment, it seems permissible to average the results and present them as mean duration-response curves (fig. 1). The latter represent typical time relations of the directional alterations seen in practically all adrenalin depressor reactions. It is apparent that changes in heart rate and blood pressure began within 8 to 9 seconds after the onset of injection and that within 12 to 18 seconds, the maximal effects were attained. With almost equal suddenness, the curves were reversed

in direction, and initial pre-injection values were obtained within the 24 to 30 second interval. For some reason, not yet apparent, the systolic pressure frequently underwent a secondary depression of lesser magnitude than the original fall. When this occurred, normal systolic values were again reached before or during the 60 to 66 second interval.

Two features of this adrenalin response merit special attention. When a dose of adrenalin, which originally elicited a depressor phenomenon, was repeated at twenty-minute intervals, the initial response was always reproduced. It appears, therefore, that the unanesthetized dog differs from the decerebrate cat in which similarly repeated injections were totally ineffective (7). The other interesting feature is the unanticipated predominant reduction of the systolic blood pressure.

Discussion. The experimental findings confirm Dragstedt's contention that doses of adrenalin under 0.2 μg./kgm. are incapable of initiating any significant elevation of arterial pressure. They are not, however, in accord with the belief that these concentrations are unable to affect the blood pressure level. Nor do they agree with the concept that doses which vary from 0.2 to 0.4 μg./kgm. are strictly minimal effective *pressor* doses. For example, a definite depressor effect was almost uniformly seen in response to 0.1 μg./kgm. quantities and a fall in blood pressure was more common than an elevation when the so-called "minimal effective pressor doses" were employed.

Despite the differences in the technical procedures adopted by the two groups of investigators, the factors underlying the discrepancies in the results are not easily determined. The rate of injection affords no clue since no correlation between rate of injection and the blood pressure response was observed. Moreover, the rates employed by the two groups were essentially equivalent in many instances. Nor can these divergencies be attributed to the selection of the carotid artery by one investigator and the femoral by the other, since it is common knowledge that, in most instances, mean pressures in the two vessels agree. The initial effects of a single injection of adrenalin should be visible in the early effects of a continuous infusion. It is surprising, therefore, that Dragstedt and his co-workers failed to detect even an initial transient fall in arterial pressure with 0.1 and 0.2 μg. quantities of adrenalin. The impression is gained from a careful examination of their published records that, at least in some instances, these reactions, which are less intense than most pressor effects, may have been partially masked by excessive respiratory undulations which were superimposed upon the non-critically damped pressure excursions as transmitted by a mercury manometer. A complete and satisfactory answer to this problem is still wanting.

The adrenalin depressor response is interesting because the dynamic mechanisms involved may be a part of the system of homeostatic buffering devices provided for bodily protection against sudden elevations of arterial blood pressure. The intimate regulation of epinephrine discharge from the adrenal medulla by the nervous system renders it not unlikely that, among other events, brief emotional disturbances may elevate circulating epinephrine to concentra-

tion levels which simulate those experimentally established in this investigation. In such circumstances, however, a reduced arterial blood pressure is not necessarily to be anticipated. Being less discrete, nature's excitatory agents may synchronously enlist other vasomotor mechanisms which are pressor in character. Consequently, the net effect of the interplay between the antagonistic mechanisms may mask any gross indications that the adrenalin depressor mechanisms are engaged at the time. Nevertheless, the dynamics of this event are worthy of further consideration.

The fall in blood pressure elicited by adrenalin has hitherto been investigated in anesthetized and decerebrate animals and has received a variety of circumstantial interpretations (12). The most widely accepted concept postulates a dilatation of smaller arteries, particularly those in skeletal muscles, and hence a reduced systemic peripheral resistance. It is understood, however, that a lessening of peripheral resistance *per se* favors a predominant reduction in diastolic pressure, providing aortic and arterial elasticity are not grossly abnormal. So far as the results obtained in this investigation are concerned, the greater diminution of the systolic pressure relegates the systemic peripheral resistance factor to a rôle of minor importance. Supplemental reasons for dismissing the latter as a cardinal participant are afforded *a*, by the brief latency between the injection and the initial circulatory response, and *b*, by the abrupt attainment of maximal effects within 12 to 18 seconds after the onset of injection.

Although positive evidence for the explanation of this reaction is unavailable from the data, further consideration reveals two hitherto neglected factors. If, for instance, the pulse pressure pattern of the adrenalin depressor effect were to be reproduced in an artificial circulation model, it would be necessary to increase the aortic capacity or to decrease the stroke output of the left ventricle.

Little is known about the behaviour of the aortic walls in the intact animal in response to adrenalin. Recently, Wiggers and Wégria (13), while eliciting an acute adrenalin hypertension in barbitalized dogs, detected an initial (probably passive) enlargement of aortic capacity. This was abruptly terminated as peak pressures were attained when a secondary diminution (probably active) set in. From this and other studies on perfused isolated sections of the aorta it is reasonable to postulate that depressor doses of adrenalin may momentarily increase the capacity of the aorta and its immediate branches in the normal unanesthetized animal.

Left ventricular stroke volume might be significantly impaired *a*, by reducing the systemic venous return; *b*, by depressing ventricular contractility, or *c*, by reducing the pulmonary venous outflow and hence the filling of the left ventricle. There is little justification for invoking the first two factors to explain this response. The third has definite possibilities. There is abundant evidence (14) in its favor though unfortunately it is derived from perfusion experiments on isolated lungs and hence must be employed with considerable reservation. Adrenalin, in quantities comparable to the depressor dose, appears to augment the pulmonary venous outflow, *if*, among other things, a constant perfusion

pressure is maintained. This effect has been attributed to a dilatation of pulmonary vessels. If adrenalin dilates pulmonary vessels when administered to the normal animal, a somewhat different series of dynamic events may be anticipated. For instance, a fall in the pulmonary arterial (perfusion) pressure will follow any significant reduction of the pulmonary resistance such as may be occasioned by dilatation of vessels. Hence, a temporary pooling of blood within this capacious reservoir must occur with a consequent reduction of pulmonary venous outflow. Accordingly the left ventricle is less adequately filled and the systolic discharge is reduced. The tachycardia which abbreviates the diastatic inflow phase of successive cardiac cycles may also accentuate this condition. The ensuing reduction in left ventricular systolic discharge is thus the most likely dynamic factor responsible for the greater diminution of systolic than of diastolic pressure in the adrenalin depressor reaction.

CONCLUSION AND SUMMARY

It appears that the unanesthetized dog will exhibit either an elevation or a depression of blood pressure in response to small intravenous injections of adrenalin, the direction depending upon the dose per unit of animal weight. The depressor reaction, which can be uniformly elicited by 0.1 μg./kgm. quantities, is reproducible in the same dog at twenty-minute intervals. Shorter intervals were not investigated. Slightly stronger concentrations of this drug may elicit either pressor or depressor effects, the latter being slightly more prevalent. Although the factors which govern the direction of the response are not completely understood, it is suspected that the initial emotional status of the animal is of considerable importance. The rate of injection does not appear to influence the direction of the response.

Since the depressor response is characterized by a predominant reduction of systolic pressure, it becomes increasingly difficult to accept the doctrine that a diminution of systemic peripheral resistance is the precipitant mechanism. We are more inclined to postulate an initial dilatation of pulmonary vessels with a consequent reduction of pulmonary arterial resistance and thus a temporary pooling of blood within the lung vessels. Hence, a transient reduction of left ventricular filling will ensue which will be further accentuated by the simultaneous tachycardia.

It has also been suggested that adrenalin may augment the capacity of the aorta and its immediate large branches. This would also exert directional effects upon the pulse pressure pattern similar to those resulting from a reduced stroke volume of the left ventricle. These two mechanisms may even act synergically in bringing about the adrenalin depression of arterial blood pressure.

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THE ANTERIOR PITUITARY IN THE CARBOHYDRATE METABOLISM OF THE EVISCERATED RAT[1]

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Evidence has been presented which indicates that there is an accelerated peripheral utilization of carbohydrate in the hypophysectomized rat and rabbit. Hypophysectomized rats lose their carbohydrate stores during fasting at a much greater rate than do normal rats. In glucose-fed rats a smaller percentage of the absorbed carbohydrate can be accounted for, four hours after feeding, in the hypophysectomized animals. In both the fed and the fasting states, the operated animals have R. Q.'s sufficiently higher than the normal to indicate that the increased carbohydrate disappearance could be due to increased oxidation (1, 2, 3). In fasted animals the apparent effect may be due to diminished glyconeogenesis in the absence of the hypophysis, but the rates of nitrogen excretion in normal and in operated rats do not differ enough to indicate that this factor is of critical importance. In the fed rats, it is unlikely that differences in rates of glyconeogenesis are a factor in producing the observed differences in carbohydrate recovery, since this would require a massive new formation of carbohydrate in the normal rats in the presence of a plethora of carbohydrate.

Greeley (4) has shown that the hypophysectomized rabbit requires the administration of large amounts of glucose to maintain normal blood sugar levels during fasting, and that this requirement, about 500 mgm. per kilo per hour, is not further increased by evisceration or hepatectomy. This requirement is considerably greater than that of the normal eviscerated rabbit which, according to Drury (5), is about 200 mgm. per kilo per hour. No attempt was made by Greeley to determine the fate of the injected glucose, but he concluded on other grounds that the extra glucose requirement was probably due to increased oxidation.

Since, as has been mentioned, previous work suggests that there is an increased peripheral oxidation of carbohydrate in the hypophysectomized rat, it seemed desirable to confirm the existence of this phenomenon in the eviscerated rat. Such a simplified preparation makes it possible not only to exclude the effects of different rates of glyconeogenesis but also to determine how far muscle glycogen deposition and lactic acid formation contribute to the differences in carbohydrate

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utilization. In the present work it is shown that in the hypophysectomized eviscerated rat there is a greater rate of fall of blood sugar than in the normal eviscerated rat, that a greater amount of glucose is required to maintain the blood sugar within normal limits, and that neither of these differences can be due to increases in muscle glycogen deposition or in lactic acid formation.

METHODS. Young male rats of the Sprague-Dawley strain, weighing from 180 to 220 grams, have been used in these experiments. The animals were not fasted, since it was desired that the hypophysectomized animals be in the best possible condition, and that the normal and hypophysectomized rats should have blood sugar levels in the same range at the beginning of the experiments.

Hypophysectomy was performed by the parapharyngeal approach 2 to 4 weeks before the evisceration experiments. The removal of the anterior lobe was complete in all cases, as judged by growth stasis and by atrophy of the testes. Adrenal-demedullation was performed always 2 weeks or more prior to hypophysectomy, in order to allow time for regeneration of the adrenal cortex. Growth rates of the rats were normal after adrenal-demedullation.

The chemical methods used were the following: for glucose, in blood filtrates and in tissue hydrolysates, the methods of Somogyi (6) or of Shaffer and Somogyi (7); for preparation of blood filtrates, Somogyi's copper tungstate reagents; for glycogen, a modification of the method of Good, Kramer, and Somogyi (8); for total carbohydrate, a modification of the procedure of West and Peterson (9); for lactic acid, the *p*-hydroxy-diphenyl color reagent, by the procedure of Barker and Summerson (10), with the Evelyn colorimeter. The latter method, with slight changes, has proven quite satisfactory in the determination and recovery of very small amounts of lactic acid.

Tail blood (0.2 ml.) and gastrocnemius muscles were used for most analyses.

Constant intravenous infusion was maintained when desired by use of a device designed and built by the Johnson Foundation, University of Pennsylvania. Special precautions were taken in the design and use of this apparatus to insure perfectly uniform infusion rates. In each of these experiments, a volume of 2 ml. per hour of fluid, consisting of 1 to 2.5 per cent glucose in 0.9 per cent sodium chloride, was infused into a femoral vein. The rate of delivery of glucose by the apparatus was determined by analysis in each experiment.

"Functional" evisceration was the procedure employed here; that is, the abdominal viscera, with the exception of the liver, were removed, and the blood vessels supplying the liver—the coeliac axis and the portal vein—were tied and cut. This operation was used, rather than absolute evisceration, because it can be performed very quickly, in 2 or 3 minutes, and without any hemorrhage or any signs of shock, even in hypophysectomized or adrenalectomized rats.

That the liver remaining in situ is effectively cut off from the blood stream is suggested by the correspondence of the survival periods and rates of fall of the blood sugar in these eviscerated rats with those in completely hepatectomized rats, determined by Selye and Dosne (11). Moreover, the lactic acid content of the asphyxiated livers of these animals may be as much as 200 mgm. per cent, with no corresponding increase in blood lactic acid. Finally, the changes in

total carbohydrate content of the livers in situ have been determined directly, by analyses of the livers in several experiments. Samples were taken immediately after the evisceration and again 2 hours later for determinations in duplicate of total carbohydrate, lactic acid, and dry weight. In six animals, with initial total liver carbohydrate of from 0.44 to 1.13 per cent, small and uniform changes occurred; the average loss of total carbohydrate was 0.062 per cent, and the lactic acid content increased 0.046 per cent. The net loss of 16 mgm. per cent corresponds to a loss of approximately 0.3 mgm. per 100 grams of rat per hour. Even these small losses disappeared entirely when the results were calculated upon a dry weight basis, since there was usually an imbibition of water by the asphyxiated livers. Since these data were obtained from animals not given glucose, in which the blood sugar was falling rapidly, opportunity for loss of carbohydrate from the liver was maximal. Therefore also in the glucose-infused animals, there was probably no contribution of the livers to the blood sugar.

The eviscerations were carried out under nembutal and very light ether anesthesia, and the animals were maintained under light nembutal anesthesia throughout the subsequent experimental periods. Rectal temperatures of all animals were maintained at 99° to 101°F. Emptying of the bladder occurred often, indicating active kidney function. No artificial ventilation was required, and the respiratory rate continued uniform throughout the course of each experiment.

Results and discussion. In the first experiments of this series, in which intact animals were eviscerated and the changes in muscle glycogen were determined, it was noticed that a marked loss of muscle glycogen occurred during the operations, an average value of 440 mgm. per cent glycogen being obtained immediately after the evisceration. A further large and variable loss occurred during the subsequent experimental periods. These changes (table 1) were considered to be the result of epinephrine secretion during and following the operative procedures, and this view was substantiated by the finding that such changes never occurred in rats from which the adrenal medullae had been removed prior to the experiments. The average value for initial muscle glycogen was 606 mgm. per cent in a series of adrenal-demedullated eviscerated rats. Changes in blood lactic acid paralleled changes in muscle glycogen. In intact rats blood lactic acid rose from 25 to 50 mgm. per cent in the hour following the evisceration. In the demedullated rats, little or no change occurred if asphyxia or convulsions from hypoglycemia were avoided: in 24 rats of all types (normal or hypophysectomized, with or without glucose) the average increase in blood lactic acid was 1.4 ± 1.1[3] mgm. per cent per hour (table 3).

From these results, it was evident that in order to study muscle glycogen changes resulting from any other causes than glycogenolysis due to epinephrine liberation, adrenal-demedullation previous to other experimental procedures was imperative. Accordingly, all the other animals used in the experiments reported here were so treated, the operation being performed 2 weeks or more

[3] Standard error.

before the experiments or before hypophysectomy, if that was carried out. It is also evident from these data that disappearance of muscle glycogen accompanied by an increase in blood lactic acid, such as is often reported to occur after evisceration or hepatectomy, need have no relation to the normal metab-

TABLE 1

Carbohydrate levels in eviscerated rats not given glucose

	NO. OF OBSERV.	SURVIVAL TIME	BLOOD SUGAR		MUSCLE GLYCOGEN	
			Initial	Decrease	Initial	Decrease
		minutes	*mgm. per cent*	*mgm. per cent per hour*	*mgm. per cent*	*mgm. per cent per hour*
1. Intact rats	6	136			440	−147
2. Adrenal-demedullated rats	12	107 ±10.2	82	−37 ±3.0	609	+2 ±11
3. Hypophysectomized adrenal-demed. rats	13	53 ± 4.5	76	−72 ±5.8	529	−108 ±16
4. Hypophysectomized adrenal-demed. rats given A. P. E.*	9	89 ± 5.4	67	−47 ±3.6	530	+1 ±13

* Saline anterior pituitary extract, 1 ml. during preceding 24 hours.

TABLE 2

Carbohydrate changes in eviscerated rats given glucose

	CHANGE IN MUSCLE GLYCOGEN DURING GLUCOSE INFUSION (2 HOURS)		CHANGE IN BLOOD SUGAR AFTER CESSATION OF INFUSION	
	No. of observ.	Mgm. per cent	No. of observ.	Mgm. per cent
1. Adrenal-demedullated rats	9	+15 ±16	12	−33 ±4.6
2. Hypophysectomized adrenal-demedullated rats	9	−49 ± 9	10	−79 ±6.1

TABLE 3

Blood lactic acid in eviscerated rats

	NO. OF OBSERV.	INITIAL LEVEL AFTER EVISCERATION	INCREASE DURING EXPERIMENT
		mgm. per cent	*mgm. per cent per hour*
1. Intact rats	6	15.7	+40.
2. Adrenal-demedullated rats	16	21.1	+1.3
3. Hypophysectomized adrenal-demedullated rats	8	16.8	+1.6
Combined data from groups 2 and 3	24	19.8	+1.4 ±1.1

olism of carbohydrate and should not be confused with the disappearance of carbohydrate due to oxidative processes.

After evisceration, if no glucose was given, the blood sugar fell rapidly in all animals, and hypoglycemic convlusions followed by death occurred when blood

glucose values of 12 to 18 mgm. per cent were approached. The survival time of the animals seemed to be determined primarily by the rate of fall of the blood sugar. As shown in table 1, the survival periods were reduced by nearly one-half by previous hypophysectomy, and the rate of fall of the blood sugar was much faster in this case than in the normal animals. At the same time, also, in the hypophysectomized (adrenal-demedullated) rats, muscle glycogen values fell about 20 per cent, whereas in the control animals no changes occurred There was no significant increase in the blood lactic acid in either instance. Previous treatment of the hypophysectomized rats with saline anterior pituitary extract lengthened the survival periods to nearly normal duration, and it also prevented the large losses of muscle glycogen. Thus, in the absence of the liver and in the absence of administered glucose, the same kinds of changes in the rate of disappearance of blood and muscle carbohydrate were affected by hypophysectomy and by injection of anterior pituitary extract as are caused by these procedures in intact fasting animals.

The rates at which it was necessary to give glucose to maintain constant blood sugar levels were determined by administering the glucose by constant intravenous infusion over 2-hour periods at various definite rates in different experiments, and determining the blood sugar at half-hourly intervals during this time. The curves obtained in such experiments, plotted as deviations from the initial blood sugar levels, are presented in figures 1 and 2. The average initial blood sugar values, determined immediately before the infusions were begun, were for the normal rats 84 $\pm$ 1.2 mgm. per cent (17 expts.) and for the hypophysectomized rats 79 $\pm$ 2 mgm. per cent (15 expts.).

From these curves it is apparent that the normal required infusion rates under these conditions lay between 13.5 and 14 mgm. per 100 grams (initial weight) per hour. Rates of from 13.2 to 19.0 mgm. per 100 grams per hour were quite insufficient in the operated rats, the animals going into hypoglycemic shock in 3 of 5 experiments by the end of the infusion periods. The required infusion rates for the hypophysectomized rats were between 25 and 30 mgm. per 100 grams per hour. These rates are comparable to those obtained by Drury and by Greeley in rabbits. They do not represent the maximum utilization rates, but rather those prevailing at blood sugar levels between 75 and 90 mgm. per cent in each case. Since the total oxygen consumption of hypophysectomized rats is about two-thirds that of normal rats, the increased rate of utilization of glucose, if it is oxidized, must represent a very much larger fraction of the total metabolism when the hypophysis has been removed than it does in intact rats.

The rates of fall of the blood sugar after cessation of the glucose infusion were also determined, and were found to be 2 to 3 times as fast in the hypophysectomized rats as in the normal animals (table 2). These figures provide further confirmation of the more rapid rate of removal of the blood sugar observed in the non-infused hypophysectomized rats.

The fate of the glucose disappearing during the infusion cannot be determined directly. In the normal animals, presumably, this glucose, or an equivalent amount of muscle glycogen, is oxidized, for it represents only a fraction of that

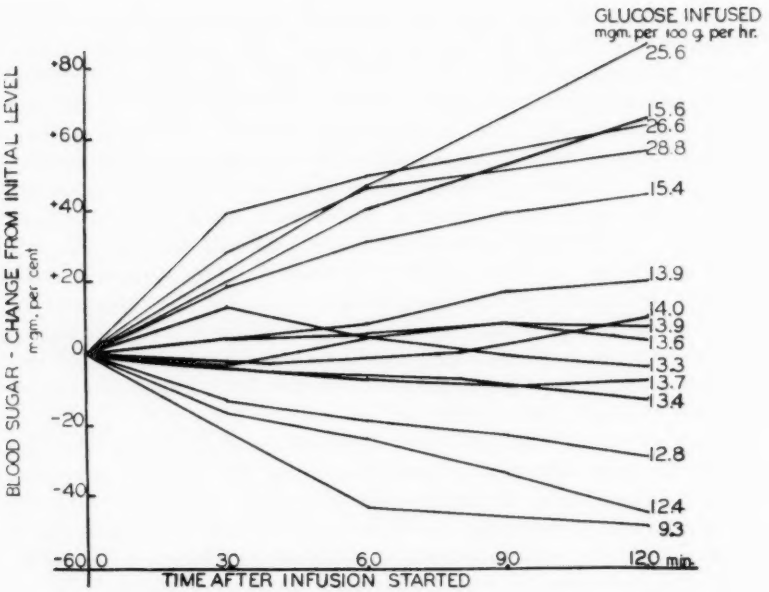

Fig. 1. Maintenance of blood sugar levels in normal eviscerated rats

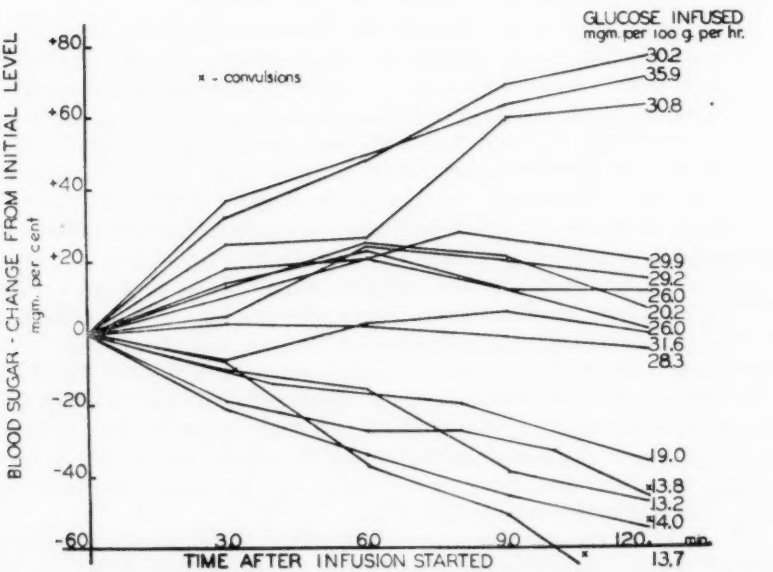

Fig. 2. Maintenance of the blood sugar in hypophysectomized eviscerated rats

required for the total metabolic needs of the animal. The increased rates of disappearance in the hypophysectomized rats were not here due to increased deposition of muscle glycogen, for the gastrocnemius muscle glycogen levels fell slightly during the infusion, while there was no change in the muscle glycogen of the normal rats (table 2). There was no increase in blood lactic acid during the infusion periods, and any large increase in the lactic acid content of the tissues should have been reflected here over the two hours' duration of the experiments. Since these two substances—glycogen and lactic acid—are the only ones in which sufficiently large changes could be expected to occur to account for the disappearance of glucose on any other basis, the carbohydrate, it can be assumed, was oxidized.

One other possible means of carbohydrate disposal remains—that of conversion to fat. However, since the liver is probably necessary in some stages of this

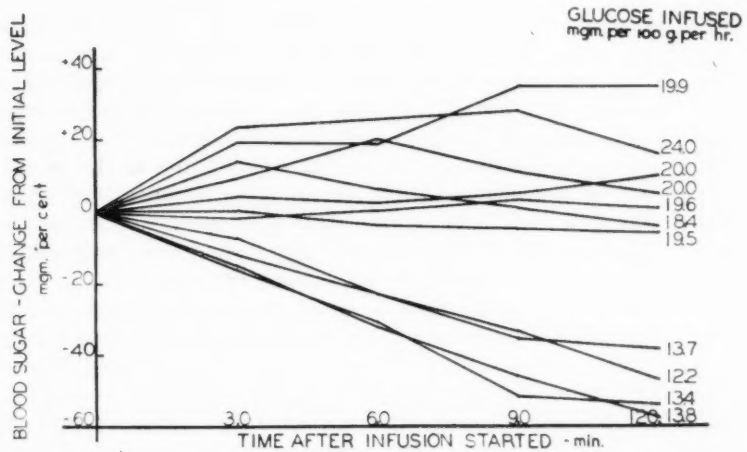

Fig. 3. Maintenance of the blood sugar in nephrectomized eviscerated rats

process, since the blood sugar levels were at no time sufficiently high to make this process likely to be rapid, and since hypophysectomized rats do not show any indications of habitual excessive fat deposition, this conversion remains an improbable explanation of this phenomenon.

The observation of Bergman and Drury (12) that nephrectomy increases the carbohydrate utilization of eviscerated rabbits suggests that defective kidney function in hypophysectomized animals might be operative in producing the results observed. The phenomenon also occurs in rats (fig. 3), but the effects of complete nephrectomy upon the glucose utilization rate were not nearly as great as those of hypophysectomy. The required infusion rate was about 19 mgm. per 100 grams per hour, and the rate of fall of the blood sugar after the cessation of the glucose infusion was 43 $\pm$ 3.0 mgm. per cent per hour (9 expts.), a value not significantly greater than the normal rate of fall (table 2).

This effect of nephrectomy on carbohydrate utilization has not been explained. Bergman and Drury suggested that the kidney normally excreted a substance which, if retained, caused increased carbohydrate utilization. However, the removal of the hypophysis, if it affects the secretory function of the kidney at all, can produce only moderate chronic defects. No signs of altered kidney function are obvious in hypophysectomized rats except the diuresis which is large immediately after hypophysectomy and sometimes persists in some degree. Urine excretion often occurred during the course of the infusion experiments and the bladders of the animals were usually full at the end of these periods. There is thus no apparent correspondence between the degree of interference with kidney function and changes in carbohydrate utilization in the two conditions of nephrectomy and hypophysectomy, and it is unlikely that the effects of hypophysectomy can be in any large part due to failure of the excretory function of the kidney.

Another explanation of the effects of nephrectomy on apparent carbohydrate utilization rates is possible: that the kidneys of eviscerated animals may normally be contributing glucose to the blood. It has been observed that kidney slices may synthesize considerable amounts of carbohydrate in the presence of amino acids, and even more from keto acids (13). With 0.025 M l(+)glutamic acid, whole kidney slices from rats formed new carbohydrate at the rate of 4.8 mgm. per gram wet weight per hour. Since there are about 0.85 gram of kidney per 100 grams weight of rat, the kidneys of the intact rat may form glucose from amino acids at the rate of at least 4 mgm. per 100 grams body weight per hour. This is a minimum value because the kidneys in situ are very likely more efficient than slices in performing such synthetic processes. It is also of the same order of magnitude as that required to satisfy the difference in the apparent utilization rates of normal and of nephrectomized rats. The blood stream is capable of supplying amino acids in sufficient quantity, since in the eviscerated animal the blood amino nitrogen concentration rises very much above the normal, to levels comparable to those used in the fluid medium of the slice experiments. Thus there is a reasonable possibility that glyconeogenesis in the kidney may play a part in determining the apparent carbohydrate utilization rate in eviscerated animals. No data is available on glyconeogenesis in kidney slices from hypophysectomized rats, but it is probably not less than in tissues from adrenalectomized rats. In the latter case, the total new formation of carbohydrate from amino acids is about 20 per cent below the normal; so that the contribution by the kidneys of the hypophysectomized nephrectomized rats would be but little less—20 per cent of the difference between normal and nephrectomized rats—than that of the kidneys of the normal rats. The large differences in utilization rates of the two series would be accounted for in very small part only by differences in glyconeogenesis by the kidneys.

The present experiments on hypophysectomized rats confirm and extend those of Greeley on rabbits. They are, however, in contradiction to those of Soskin and his colleagues, who worked on dogs (14). The method of measuring carbohydrate utilization used by the latter investigators has been criticized

previously by Chambers and Barker (15) and by Cori and Cori (16). In this method, the decline in body glycogen (calculated from individual muscle glycogen determinations) was added in each case to the amount of glucose infused, and this sum was corrected by the subtraction of increases in the glucose and the lactic acid content of the body (calculated from changes in blood levels). In cases where moderate amounts of glucose were given and blood sugar levels were maintained within normal limits, the major contributions to the final figure obtained for carbohydrate utilization were made by the losses of muscle glycogen. Since these losses were always accompanied by elevation of the blood lactic acid values, they were undoubtedly the result of glycogenolytic processes not necessarily reflecting oxidation of carbohydrate. In the hypophysectomized dogs, Soskin and his co-workers reported that changes in muscle glycogen and blood lactic acid were much smaller than in the normal dog; consequently the utilization figures calculated from these data indicated low or normal utilization rates. Sufficient data are not given to make clear the reason for this difference in glycogenolysis; it may have been due, for instance, to such a non-specific cause as a difference between the two series of animals in their relative ventilation rates, normal dogs requiring more oxygen than the hypophysectomized animals and so exhibiting asphyxial glycogenolysis at ventilation rates which were still sufficient for the operated dogs. In any case, it does not seem profitable to compare utilization rates of carbohydrate calculated upon the basis of changes in muscle glycogen which may not have been related to the oxidation of carbohydrate. It will be necessary to reinvestigate this problem before invoking species differences between rabbits and rats, and dogs, to explain the differences in the apparent effects of hypophysectomy upon the carbohydrate metabolism of these animals.

SUMMARY

Normal, adrenal-demedullated and hypophysectomized rats have been compared in their metabolism of carbohydrate in the absence of the abdominal viscera. Removal of the adrenal medullae previous to the evisceration prevented the occurrence of any significant changes in muscle glycogen and in blood lactic acid during or following the evisceration in normal rats, if asphyxia or convulsions were avoided. All animals used in other experiments were then adrenal-demedullated before hypophysectomy or before evisceration was performed, and in these experiments, therefore, glycogenolysis due to epinephrine liberation was avoided.

Hypophysectomized demedullated rats after evisceration survived only half as long as normal demedullated rats and utilized their blood glucose at two to three times the normal rate. Their muscle glycogen also fell moderately during the survival period. This fall was prevented, and the survival time was prolonged, by previous treatment of the rats with saline anterior pituitary extract. There was no increase in blood lactic acid during the survival periods.

Hypophysectomized rats required glucose administration at the rate of 25 to 30 mgm. per 100 grams per hour to maintain normal blood sugar levels, whereas

the control rats required only about 13.5 mgm. per 100 grams per hour. This increased requirement of glucose was not due to deposition of muscle glycogen or to liberation of lactic acid; it was therefore presumed to be due to increased oxidative utilization of carbohydrate in the peripheral tissues, in the absence of the anterior pituitary gland.

Nephrectomy increased moderately the need for glucose in rats, as it does in rabbits. Kidney damage is not indicated to be an important factor in producing the increased utilization of carbohydrate in hypophysectomized rats.

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HEAT EXCHANGES DURING RECOVERY FROM EXPERIMENTAL DEFICIT OF BODY HEAT

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The adjustments whereby the temperature of the human body remains constant may be considered as consisting in: *1*, loss of heat when the temperature is too high, and *2*, gain of heat when the temperature is too low. But since heat is continuously both produced and dissipated, net loss may result by slowing the production as well as by accelerating the dissipation. Much evidence indicates that little is done by the overheated man to decrease production, and compensation consists in faster dissipation (Adolph, 1938). What is the situation in the undercooled man? Is body temperature restored by faster heat production (chemical regulation), or by slower heat dissipation (physical regulation), or by both? What part does evaporation of water play in the modification of heat losses?

Quantitative answers to these questions depend on the measurement of changes in heat content of the human body. A reading of temperature in the rectum is sometimes considered to indicate what excess or deficit of body heat exists. It is well known, however, that undercooled parts of the human body are often isolated from interchanging heat with other parts at the usual rates. In the present research the temperatures of many localities are measured, and from them integrated temperatures of the body are estimated. These are converted into equivalent heat contents; thereby the heat content of the body is obtained in units commensurate with those of heat exchange.

PROCEDURE. Several methods of inducing deficits of heat content have been tested. The unanesthetized human body easily defeats most efforts to deplete it of heat. Immersion in cold water was studied calorimetrically by Lefèvre (1911); he showed that the rates of heat loss diminish with each minute of exposure. Exposure to cold air cooled the body somewhat in the tests of Lefèvre, Swift (1932), and others. We found that ice packs, ice-water irrigation of the rectum, and cold shower baths cooled local portions of the body without concurrently diminishing the temperature of the remainder; they did not allow the rapid induction of measurable heat deficits. We therefore drank ice-water.

Body heat production, heat content, heat loss by evaporation of water, and heat loss by radiation and convection were measured during the recovery from

[1] Aided by a grant from the Fluid Research Fund of this School. We are indebted to Dr. S. W. Clausen for facilities and counsel.

each deficit produced. Thus the part played by the several factors in paying off the heat debt could be evaluated at all times during the experiment and could be related to the moving heat debt.

A brick room, insulated on the windowed side by an extra partition of wallboard, was used for the tests. During the experiments the air of this room was kept at 31°C. ±1° and 20 to 30 per cent relative humidity. The nude subject occupied a reclining seat on a Sauter balance. Rates of non-renal water loss were measured by weighing the man at intervals. Urine collected periodically was kept in a stoppered flask on the balance. Heat production was estimated by means of a Benedict-Roth oxygen consumption apparatus. Rectal temperature was read to ±0.01°C. on a thermometer inserted to a depth of approximately 5 cm., the stem of which projected through a slit in the seat. Readings could thus be taken throughout the test without altering the position of the thermometer. Mouth temperatures were taken by clinical thermometer. Surface temperatures were determined on twelve representative areas with a movable, fine, copper-constantin thermocouple, and then weighted according to the extent of each area represented, to obtain mean surface temperature. The pulse frequency was recorded. Room temperature and relative humidity were ascertained by thermometer and calibrated hair-hygrometer respectively.

The subject entered the room 2 to 5 hours after a light breakfast or no breakfast, undressed and sat on the balance. After 15 to 20 minutes, to allow for partial equilibration of the body with room conditions, a control period of 30 to 50 minutes was inaugurated, during which observations were made at approximately 20-minute intervals. Then the subject drank 1.4 to 1.7 liters of water at 1° to 3°C., taking 10 to 14 minutes for its ingestion, and observations were continued, at as short intervals as possible (15 to 25 min.), for 150 to 210 minutes.

Heat loss by evaporation. The weight loss recorded by the Sauter balance represented water evaporated from the body plus carbon lost as carbon dioxide. Since the carbon constituted only 5 to 10 per cent of the total weight loss, no correction for this factor has been made; the total has been treated as loss due to evaporation of water. This water loss has been translated into heat loss by use of the factor 0.58 Cal./gram for latent heat of vaporization of water at 37°C. If a correction for the carbon loss were applied, it would reduce the values for rates of evaporative heat loss by approximately one Calorie per square meter per hour (fig. 1).

The vaporization of water in the control period, while quite variable on different days, accounted on the average for approximately 50 per cent of the heat flowing out of the body. This percentage is probable evidence of slow sweating, at diverse rates. Following ingestion of the cold water, the heat loss by vaporization diminished to only 25 per cent of the total production of heat in the body, a figure which agrees closely with the ratio found by Benedict and Root (1926) and others who studied subjects in cooler environments and in basal states. These minimal rates of vaporization in our experiments prevailed for about 80 minutes after ingestion of the cold water; and the rates on different days agreed closely (fig. 1). By 180 minutes the rates of vaporization had reattained their

pre-ingestion magnitudes. The reduction in rate of loss by vaporization is sufficient to account for 50 to 55 per cent of the heat regained by the body during the 180 minutes of recovery.

Heat production varied as much as 16 per cent from the mean in some subjects, but showed no consistent change after cold water was drunk. It took little, if any, part, therefore, in the recovery from the heat deficit. This conclusion is also suggested by data of Winslow *et al.* (1937) who found no significant alteration in the rate of heat production over a wide range (16° to 42°C.) of environmental air temperatures, even though large heat deficits (1.0 to 1.5 Cal. per

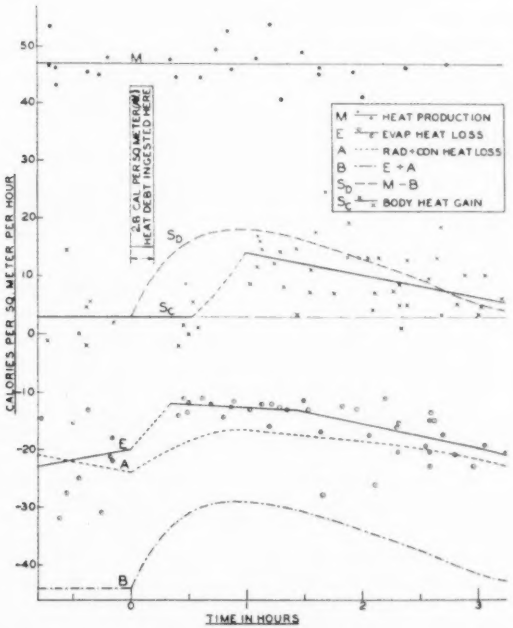

Fig. 1. Body heat exchanges following a heat debt produced by ingestion of 1540 ml. of water at 1° to 3°C. in an environmental temperature of 30.9°C. (6 experiments on 4 subjects).

kgm.) resulted when the subjects were exposed to the colder airs. Loewy (1890) and others reported that heat production was not increased by external cooling, except when shivering or other minor muscular activity resulted. Hardy *et al.* (1941) found moderate increases in rate of heat production in women but not in men exposed to cool air.

Cannon *et al.* (1926) in 22 tests on eleven clothed subjects at an environmental temperature of 20°C. found that ingestion of sufficient ice water to produce small heat debts, of approximately 0.45 Calorie per kilogram of body weight, induced an average maximal increase of 10 per cent in heat production in those experiments where shivering was absent. When shivering occurred, brief increases in

heat production up to 90 per cent above basal were recorded. Cannon *et al.* suggested that at higher environmental temperatures such rises in heat production might not be observed.

At the air temperature of 31°C. our rates of heat production varied to the same degree as those of Cannon's subjects who did not shiver. Slight gooseflesh associated with an urge to shiver was felt by two of our subjects. However, actual shivering occurred in only one test; it was slight and of short duration. In the test in which the rate of heat production increased most, the additional heat supplied was sufficient to pay off only 15 per cent of the heat deficit induced.

TABLE 1

Temperature alterations following water ingestion in an environmental temperature of 31°C.

DATE OF EXPT., 1939	SUBJECT	WT.	WATER DRUNK	TEMP. OF INGESTED WATER	MAXIMAL TEMPERATURE DEFICIT			CALCULATED DEFICIT IN MEAN BODY TEMP.	RATIO OF DEFICITS OF MEAN SURF. TEMP.: RECTAL TEMP.	TIME AFTER INGESTION OF LOWEST RECORDED TEMP.			TEMP. DEFICIT 150 MIN. AFTER INGESTION		
					In rect. temp.	In mean surface temp.	In mean body temp.*			In rectal temp.	In mean surface temp.	In mean body temp.	In rectal temp.	In mean surface temp.	In mean body temp.
Water ingested at 2°C.															
		kgm.	*ml.*	°C.	°C.	°C.	°C.	°C.		*min.*	*min.*	*min.*	°C.	°C.	°C.
2/1	E. A.	76	1445	3.5	0.90	0.30	0.69	0.72	0.33	68	70	70	0.32	−0.15	0.16
2/6	E. A.	76	1541	2.5	0.73	0.84	0.76	0.80	1.15	56	56	56	0.12	0.57	0.26
1/23	E. P.	65	1710	1.0	1.02	1.49	1.08	1.10	1.44	40	86	48–61	0.25	1.27	0.59
2/13	E. P.	66	1415	1.5	0.72	0.83	0.73	0.87	1.15	42	66	42	0.22	0.07	0.16
2/19	H. K.	79	1649	2.5	0.70	0.41	0.60	0.82	0.59	57	57	57	0.14	−0.15	0.04
2/18	M. P.	72	1482	1.0	0.82	1.10	0.92	0.85	1.34	60	51	51	0.47	0.61	0.54
Average.....			1542		0.81	0.83	0.80	0.86	1.02	54	64	55	0.25	0.37	0.29
Controls—water ingested at 35°C.															
2/8	E. A.	76	1527	35.0	0.15	0.16	0.11	0	1.07	67	37	37–67	0	−0.22	−0.08
2/15	E. P.	66	1423	36.5	0.11	0.42	0.20	0	3.85	60	60	60	0.03	0.06	0.04
Average.....			1475		0.13	0.29	0.16	0	2.44	64	48	56	0.01	−0.08	−0.02

* Calculated from recorded deficits of rectal and mean surface temperatures.

Rectal temperature attained an average maximal decrement of 0.81°C. Fifty-four minutes (average) after cold ingestion were required to reach this maximum, but the time varied as much as 14 minutes from this mean in individual tests. The curves for rectal temperature against time descended smoothly, and subsequently rose gradually, the rate of increase being proportional to the displacement of the rectal temperature below the initial. At the end of 150 minutes after the ingestion, the rectal temperature had recovered two-thirds of this deficit; the average deficit was at this time only 0.25°C. (table 1).

Surface temperature. The mean temperature of the body surface suffered a maximal decrease of 0.83°C. (average), which equals the average maximal dec-

rement of rectal temperature. However, the ratio of maximal decrease of surface temperature to maximal decrement of rectal temperature in individual experiments varied from 0.33 to 1.5 (table 1). The surface temperatures were lowest at 64 minutes (average), or 10 minutes after rectal temperatures were lowest. At 150 minutes the surface temperatures had recovered one half of the deficit and were within 0.37°C. of the initial value. Surface temperatures tended to remain low until the body had recovered some of the heat deficit (table 2). They did not rise at rates strictly proportional to their displacement below the initial, differing from rectal temperature in this respect. Rates of increase of surface temperatures were greatest between 150 and 200 minutes, at a time when rates of increase of rectal temperaure had fallen off to relatively small values. By waiting until considerable recovery of rectal temperature had taken place, surface temperatures acted to conserve heat production by reducing heat loss

TABLE 2

Rates of temperature change at various times following ice-water ingestion

MINUTES AFTER INGESTION	TEMPERATURE CHANGE DURING THE INTERVAL, IN °C.			RATE OF TEMPERATURE RISE IN °C. PER HR.			PER CENT OF RECOVERY AT END OF EACH INTERVAL		
	Rectal temp.	Mean skin temp.	Mean body temp.	Rectal temp.	Mean skin temp.	Mean body temp.	Rectal temp.	Mean skin temp.	Mean body temp.
0–30	−0.66	−0.59	−0.60						
30–60	−0.15	−0.24	−0.20						
60–90	+0.22	+0.10	+0.15	0.44	0.20	0.30	27	12	19
90–120	+0.20	+0.15	+0.19	0.40	0.30	0.39	52	30	43
120–150	+0.11	+0.17	+0.15	0.22	0.34	0.29	66	51	61
150–180	+0.10	+0.18	+0.12	0.20	0.36	0.24	78	73	76
180–200	+0.04	+0.12	+0.06	0.12	0.36	0.18	83	87	85

Averages of the six experiments at an environmental temperature of 31°C.

through radiation and convection. The part played by this lag in the make-up of the temperature deficit will be discussed below.

Oral temperature, which was ordinarily about 0.5 to 0.6°C. below rectal temperature, fell sharply when ice-water was taken through the mouth and did not reassume its position relative to rectal temperature until about 60 minutes after ice-water ingestion. Thereafter it rose as the other body temperatures rose.

Decrements of surface temperature tended to be greater on the extremities than on the trunk. Later the surface temperature of the extremities increased more slowly than that of the trunk region; thus the surface temperatures of arms and legs were the last to recover.

Mean body temperature and heat content. The mean body temperature was derived from measurements of the rectal temperature and mean surface temperature with the use of the following formula (Burton, 1935): $\frac{2}{3}$ rectal temp. + $\frac{1}{3}$ mean surface temp. = mean body temp. Thus computed, the average maximal fall of the body temperature was 0.80°C., while the average time that it took this fall to occur was 55 minutes. This represents the time for the heat

deficit, contained in the water ingested, to become evenly distributed through the body. Sixty per cent of this deficit had been recovered at 150 minutes (table 2), and 85 per cent at 200 minutes.

The amount by which the mean body temperature would have decreased if the distribution of the heat debt through the whole body had taken place immediately and equitably, is calculated in the following manner: $\Delta T = W(T_b - T_w)/(W + 0.83\ B)$, where T_b is the initial mean body temperature, T_w is the temperature of the water on its ingestion, W is the amount of water ingested in kilograms, B is the body weight in kilograms before ingestion, and 0.83 is the specific heat of the body. ΔT thus calculated for individual experiments is recorded in column 9 of table 1.

Interestingly enough, the calculated temperature fall exceeds but little the actual fall found 55 minutes after the beginning of ingestion (column 8). Only 7 per cent of the heat debt seems to have been paid off within this period of time.

Heat loss by radiation and convection was computed as follows. *a*. Total heat loss equalled heat production minus heat retained or stored. Total heat loss minus evaporative heat loss was then heat loss by other means (radiation, convection, conduction). Alternatively, *b*, the heat loss by these other means during the pre-ingestion period having been ascertained, this fraction of heat loss in the subsequent periods was assumed to be directly proportional (Gagge, 1936; Winslow *et al.*, 1937) to the difference between mean surface temperature and air (and wall) temperature. Such calculations show that 9.0 Calories per square meter per hour flowed from the body for each 1°C. difference between skin and environment, during the pre-ingestion period of these experiments. (This figure is 9.4 if we correct the evaporative heat loss for the carbon exhaled as previously discussed.) Winslow, Herrington and Gagge's (1937) data show a radiation and convection loss rate of only 7.0 Calories per square meter per hour per degree C. difference between skin and environment. This discrepancy of 2.0 or 2.4 Cal. is most likely due to a difference in convection in the two experimental conditions. Turbulent air movement in their experiments was 5 to 6 meters per minute; air movement of 14 meters per minute in our experiments would account for the discrepancy noted. While we did not measure air movement, the velocity mentioned is considered likely.

Total heat exchanges. In figure 1 the sum of heat loss by vaporization and of heat loss by radiation and convection (*b*) is plotted as total heat loss. This total heat loss when subtracted from heat production gives the curve for heat storage in the body, designated by S_D. Another curve for heat storage, designated S_C, is a mean of the rates of heat gain by the body as indicated by the rise in mean body temperature during intervals in each test. The points plotted between 0 time and 60 minutes, a period when the mean body temperature was actually falling, however, were determined by subtracting the recorded fall in body temperature from the fall in body temperature (ΔT) which the water as ingested was calculated to produce (table 1).

The average heat debt induced in the body computed from ΔT was approximately 53 Calories. The debt paid off under curve S_D represented approxi-

TABLE 3

Recovery from a deficit of body heat in an environmental temperature of 28°C.

TIME	RECTAL TEMP.	MEAN SKIN TEMP.	MEAN BODY TEMP.	MEAN BODY TEMP. DEFICIT	HEAT PRODUCTION	NON-RENAL WEIGHT LOSS	URINARY EXCRETION RATE
	°C.	°C.	°C.	°C.	*Cal./sq. m./hr.*	*gm./hr.*	*ml./hr.*
7:50– 9:38							28
9:16– 9:43						56.6	
9:23					40.0		
9:30	37.48	33.50	36.15				
9:43–10:04						56.6	
9:48					43.7		
9:52	37.50	33.36	36.12				
10:04–10:54						36.1	
10:10					40.0		
10:15–10:41	Ingested 1468 ml. of water at 1.0°C.						
10:20	37.42			(0.08)			
10:40	37.22			(0.28)			
10:43	37.16	32.59	35.64	0.48			
10:54–11:12						35.1	
10:57	36.83			(0.67)			
10:58					38.1		
9:38–11:14							82
11:04	36.75			(0.75)			
11:12–11:31					38.9		
11:14	36.68			(0.82)			
11:14–11:36							493
11:18					39.4		
11:31–12:01						30.3	
11:36	36.61			(0.89)			
11:36–12:08							510
11:49	36.62	32.42	35.22	0.90	41.8		
12:01–12:26						37.6	
12:03	36.71	32.45	35.29	0.83			
12:14					40.0		
12:08–12:29							565
12:18	36.80	32.55	35.38	0.74			
12:26–12:56						33.7	
12:29–12:48							538
12:33	36.90	32.46	35.42	0.70			
12:48– 1:02							407
12:48	36.90	32.38	35.39	0.73			
12:56– 1:14						31.4	
1:03	36.91	32.57	35.46	0.66			
1:02– 1:23							300
1:16	36.90	32.70	35.50	0.62			

Subj., A. W. Wt., 69 kgm. S. A., 1.85 sq. meters. Aug. 9, 1939. Expected deficit of mean body temperature (ΔT), 0.88°C.

mately 73 Calories. These computations suggest that the decrease in total heat loss after ingestion is somewhat exaggerated by curves S_D and B, and that the heat exchange represented by curve S_C is the more accurate one.

In general, the deficit of heat was replaced in the body not by augmented production of heat through oxidative metabolism, but through diminished loss of heat. Half the diminution was in evaporative loss and half was in radiative loss. Recovery was half complete in 120 minutes and wholly complete in approximately 220 minutes after the deficit arose.

In control experiments, in which water at average body temperature was ingested, slight decreases in mean body temperature (0.11 to 0.20°C.) resulted. The heat deficit produced in this manner was completely paid off in 150 minutes after drinking (table 1).

Pulse frequency. Following ingestion of cold water the pulse frequency decreased sharply, reaching its minimum between 20 and 30 minutes after drinking began. From about 9 beats per minute below its initial frequency, the rate gradually increased until at 180 minutes it was only 4 beats per minute below the initial (fig. 2). In the control experiments the pulse frequency also diminished, but more gradually and less.

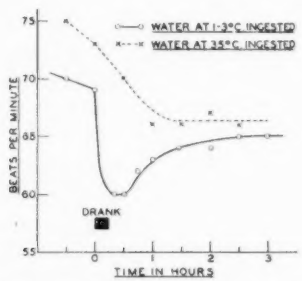

Fig. 2. Change in pulse rate after ingestion of 1540 ml. of water. (Averages of 5 + 2 experiments on 3 subjects.)

The sharp decline in pulse frequency following ice-water ingestion may be due to conduction between the large mass of cold water in the stomach and the heart. The position of the stomach when filled with cold water, could be felt as a cold area on the external abdominal wall.

No correlation between pulse frequency and oxidative metabolic rate could be noted.

Rate of urine excretion. The diuresis which resulted from the ingestion of 1540 ml. of water was sometimes delayed when the water was taken cold (fig. 3). In one subject the diuresis appeared 65 and 90 minutes later than it did after a control ingestion of the same amount of water at 35°C. (E.F.A., fig. 3). The maximal rates of urine excretion after cold water ingestion were often less than after an equal amount of water at 35°C. was drunk. Thus the ingestion of cold water diminished the maximal rate of excretion as well as delayed the diuresis; both are phenomena which might represent deferred absorption of the cold water from the alimentary tract.

Effect of air temperature on recovery from body heat deficit. One experiment was

conducted in air at 28°C., in the same manner as those at 31°C. previously described. A condensed protocol of the results (table 3) shows that recovery from the heat deficit at 28° is much slower and less complete than in the warmer air. In 180 minutes after ingestion of the water only 31 per cent of the maximal deficit had been paid off, and recovery as indicated by rectal temperature was at a standstill. In an equivalent length of time in 31°C., 76 per cent of the deficit was recovered and all temperatures were still rising (table 2).

At environmental temperatures higher than 31°C. an induced heat deficit might be discharged more rapidly than at 31°C., since current loss of heat could be still further diminished.

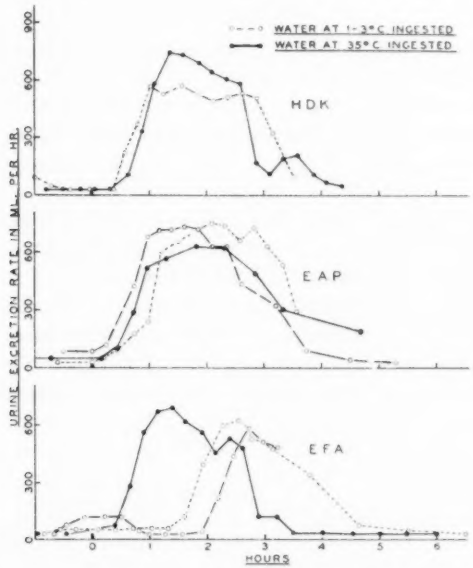

Fig. 3. Diuresis produced by ingestion of 1540 ml. of water, begun at zero time and completed within 10 to 14 minutes. Note delay in excretion in last subject occasioned by ingestion of cold water (broken-line curves).

Since even in air at 28°C. no significant alteration in heat production was found after ingestion of the cold water, all the recoveries here recorded are to be attributed to decrease in the rate of heat loss. In other subjects, differently acclimatized, an increase of heat production might be found.

Comment. Having been taught that extra heat production (chemical regulation) occurred when the human body was too cool, we were interested to find that in the conditions of our experiment such was not the case. Instead, heat loss was reduced so that no extra energy was expended in making up the heat deficit.

The occurrence of "chemical" regulation in man has been demonstrated by several early workers (Yagloglou, 1924). Hardy *et al.* (1941) showed that women exposed to cold air produced heat appreciably faster; men did not. But *re-*

covery from cooling has been studied only in the discontinuous measurements of Cannon *et al.* (1926), who found extra heat production in several subjects; the significant increments seem to have accompanied shivering.

Possibly shivering and augmented heat production occur more often with cool skin. Our adult subjects did not compensate for heat deficit by augmenting heat production; the same individuals may have had the ability to do so in infancy, and may be able to acquire it again after acclimatizing exposures. If chemical regulation acts in more extreme deficits of heat, or in deficits of particular kinds, or in certain states of acclimatization, these contingencies need to be demonstrated for man.

SUMMARY

Heat exchanges were measured in four men who sat unclothed in air at 31°C. and 25 per cent relative humidity. In each test the body became cooler by 0.72 Calories per kilogram when 1.5 liters of ice-water were drunk. The mean heat content was estimated from the temperatures of many regions of the body at various times thereafter.

In 200 minutes, 85 per cent of the initial heat debt was paid off. Half of the heat regained came from the reduction in loss by vaporization of water. The other half came from the reduction in loss by radiation and convection, concomitant with the diminution of surface temperatures.

Heat produced by oxidations was not augmented. No shivering occurred.

At a lower environmental temperature (28°C.) the recovery was slower.

It was noted that the heart frequency was diminished after ice water was drunk, and that the ensuing water diuresis was sometimes delayed.

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PRESSURE OF BLOOD IN THE RIGHT AURICLE, IN ANIMALS AND IN MAN: UNDER NORMAL CONDITIONS AND IN RIGHT HEART FAILURE[1]

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It has long been known that the pressure of blood in the right auricle of a normal anesthetized mammal, lying supine, varies around a mean value slightly less than zero or atmospheric (1, 2). The pressure in the great veins, as would be expected, shows a gradual increase as one passes peripherally.

There has been, however, little study either of the right auricular or of peripheral venous pressures in animals in conditions of right heart failure.

No measurements of right auricular pressures have previously been recorded in man, either under normal conditions or in heart failure, so far as we are aware.

I. *Venous and auricular pressures during right heart failure in animals.* Right heart failure in an animal was first encountered accidentally. Pressures were being recorded by means of saline manometers in arm, leg, and right auricle of a large chimpanzee. These results are shown in figure 1. After the experiment had proceeded for about an hour and a half, a needle was inserted into the pleural space to obtain a record of pleural pressure. The animal rapidly developed pulmonary edema. At the same time both auricular and peripheral venous pressures rose and both became nearly equal. These phenomena clearly indicated an acute heart failure, the rise of venous pressure indicating the degree of "backward failure."

Autopsy of the animal showed moderate pulmonary edema. There was no evidence of injury to the lung from the intrapleural needle.

A study of the same phenomena was subsequently undertaken, using dogs. The technique was similar except that right auricular pressure was recorded by long rubber catheters passed into the right auricle either from below via femoral vein or from above via the jugular.

A preliminary question concerned the reliability of the technique of using long catheters of narrow caliber (no. 6 to no. 10) passed along the great veins, for recording of central venous pressure.

a. It was easily shown that a catheter could be passed into the femoral vein up

[1] A preliminary report of a part of the material was presented at the Association of American Physicians, May, 1941.

[2] Working under a grant from the Commonwealth Fund.

the vena cava to the auricle, and withdrawn again, with no measurable effect upon the pressure in jugular vein, in right auricle (as measured by another catheter extending down from the jugular vein), or in the opposite femoral vein. There appeared, therefore, to be no effects due to obstruction or spasm of vessel walls.

b. There was no significant error due to capillary effects within the catheter. The levels of both saline manometers were too high by 4 mm., by reason of the capillary effect at the meniscus. Correction was made for this error.

c. The changes in pressure with the animal's respiration, however, were not recorded accurately by the long intra-auricular catheters. This was to be expected, since to record such changes involved fairly rapid flow of fluid through the catheter, with each inspiration and expiration.

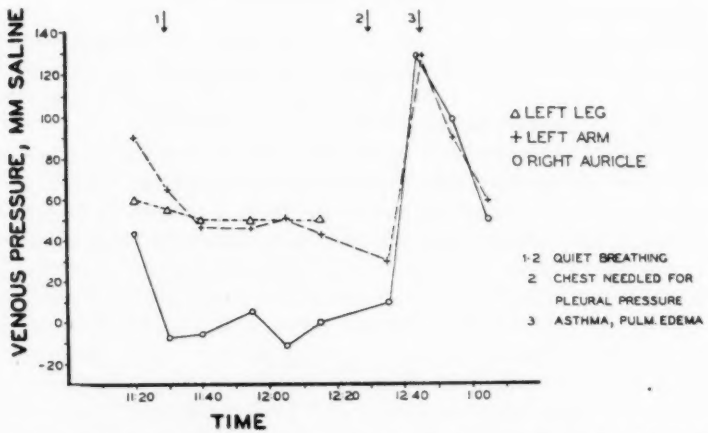

Fig. 1. Venous pressures, in millimeters of water, in the arm, leg and right auricle of an anesthetized (nembutal) chimpanzee.

The actual expiratory levels of venous pressure could be obtained separately, simply by shutting off the connection between catheter and manometer during inspiration, opening it only during the phase of expiration. Equilibrium was established slowly in this way, after perhaps a dozen respirations. Shutting off the connection during expiration similarly gave the inspiratory venous pressure level. It was found that the pressure recorded when the manometer was left connected continuously with the right auricle was always the lowest or inspiratory pressure, saline being more readily drawn down the catheter during inspiration, than blood returned into it during expiration.

Figure 2, describing one of several similar experiments on dogs, gives the essential results. It will be seen that the deep labored respiration of tracheobronchial obstruction increased greatly the pressure differences between inspiratory and expiratory states, in the right auricle.

For practical purposes it was found that with dogs anesthetized by nembutal,

the use of an intratracheal cannula prevented obstructive respiration and maintained inspiratory-expiratory pressure differences near minimum values.

For the study of right heart failure (associated with acute pulmonary edema), we used the technique of Miller and Matthews (3), producing pulmonary edema

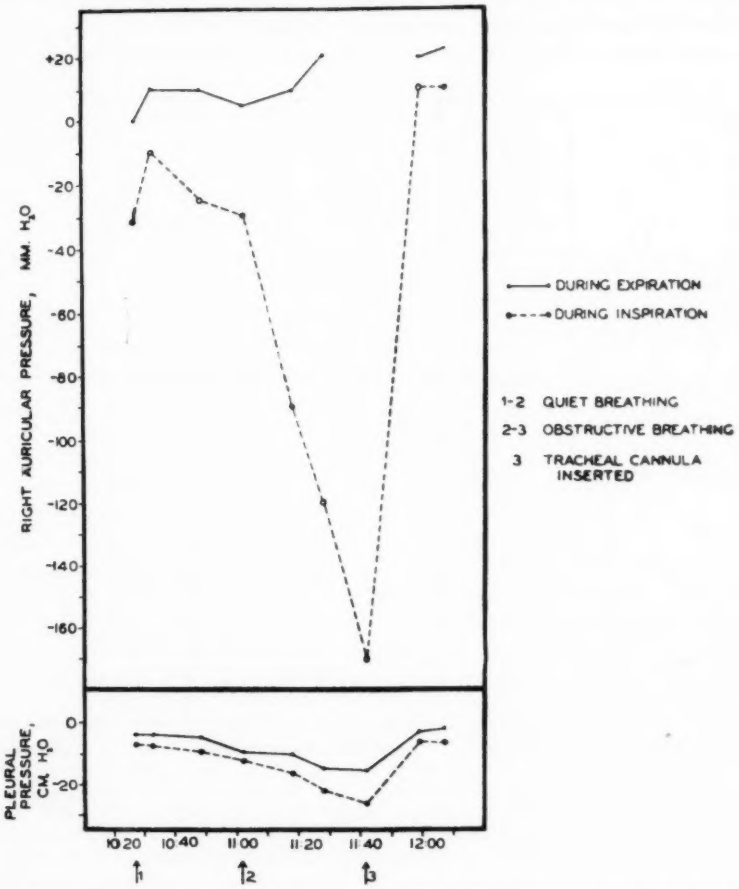

Fig. 2. Anesthetized dog (nembutal) showing the effects of spontaneously developing respiratory obstruction, upon the inspiratory and expiratory phases of right auricular and pleural pressures.

by inhalation of ethyl acetate vapor. Figure 3 gives the results of four such experiments.

It was found that by adjusting the concentration of vapor inhaled, pulmonary edema could be brought on relatively rapidly—in 15 to 30 minutes—or very gradually. In the third experiment shown in figure 3, a low concentration of ethyl acetate vapor was inhaled continuously for 83 minutes.

All animals at autopsy showed dilated right auricles and pulmonary edema, the latter demonstrated grossly and microscopically. Dogs 4, 5 and 6 died as a direct result of the ethyl acetate administration; dog 7, given the lower concentration of the gas, while still in a relatively early stage of right heart failure, and with arterial pressure maintained, was killed by intracardiac chloroform.

The chief finding in these experiments confirms that of the chimpanzee experiment; namely, that with development of right heart failure both peripheral

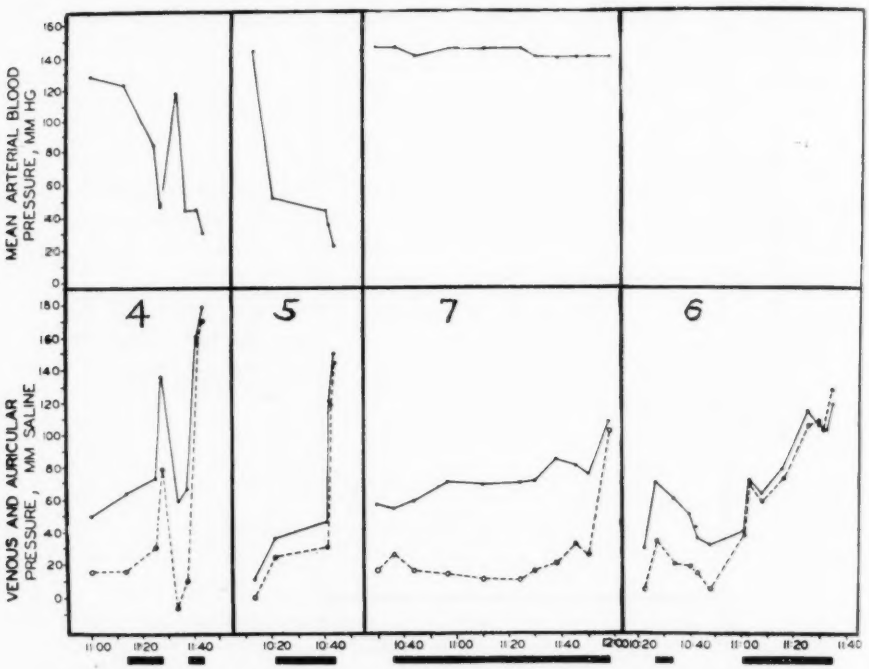

Fig. 3. Effects of inhalation of ethyl acetate vapor, upon arterial, venous, and right auricular pressures, in a series of 4 dogs. Upper curves: mean arterial pressures. Lower curves: pressures in femoral vein (solid lines), and right auricle (broken lines). Solid blocks below indicate periods during which ethyl acetate was inhaled.

and central venous pressures rise and the gradient between peripheral and central pressures decreases almost to the vanishing point.

II. *Pressure in the right auricle of man, under normal conditions and in right heart failure.* Catheterization of the right heart in man was first essayed by Forssmann (4), and has subsequently been used for various studies (5, 6, 7, 8, 9, 10). The usual technique is to insert a long (70 cm.) ureteral catheter (no. 7 or no. 8 French) into a median basilic vein, then pass it along brachial, axillary, and subclavian veins until (under fluoroscopic observation) the tip is seen to lie in the right auricle. Cournand and Ranges (11) have recently developed improve-

ments in certain details of this technique, and have used right heart catheterization in man for determination of cardiac output by the direct Fick principle. These investigators showed that right heart catheterization caused but slight changes in circulation, respiration, and respiratory exchange; notably, a slight increase in minute ventilation and a slight slowing of the pulse rate.

In the present study, we have used the technique of right heart catheterization for recording venous pressure, in nine normal subjects and in six patients with heart disease, three of whom were in congestive failure, the other three recovering from this condition. The general procedure was as follows:

The preparation of the arm and the insertion of the catheter were carried out with the subject lying in his bed. When the catheter had been passed successfully into the axillary vein, and a slow flow of saline (15 drops per min.) from a reservoir was established through the catheter to maintain its lumen and prevent clot formation, the subject was transferred to the fluoroscopy table, and the catheter then pushed on into the right auricle under fluoroscopic observation. Anteroposterior and lateral x-rays were usually taken. By these x-rays, a record of the catheter's position was made, and the absolute level of the catheter in reference to the outer chest wall could be determined. The subject then was transferred back to his bed, supine with head very slightly elevated, and the catheter was connected with a saline manometer. Peripheral venous pressure was recorded in the usual manner by the direct method, with a needle inserted in a vein of the other arm, and connected with a second manometer. The zero of both manometers was taken arbitrarily as a point 5 cm. below the angle of Louis on the external anterior chest wall. In the cases where x-rays of the catheter were made, the manometer figures were subsequently corrected according to the actual position of the catheter in the chest, as will be discussed later.

A series of observations of peripheral venous and of right auricular blood pressures was then carried out. On several occasions, the catheter was withdrawn by stages, thus affording opportunity to measure pressures in superior vena cava, subclavian and axillary veins. The gradient of pressure appeared to be nearly linear, as might be expected. It should be noted that during such withdrawal of the catheter, there was no change in the pressure recorded in the vein of the opposite arm, thus indicating that the procedure itself probably does not disturb venous pressure levels.

The respirations of the subjects, both normal and cardiac, were quiet and easy during the procedure. It did not seem likely that there would be large inspiratory-expiratory differences in auricular pressure. In two subjects, expiratory pressures were recorded separately, by shutting off the manometer during each inspiratory phase. It was found that there was less than 10 mm. difference between inspiratory and expiratory pressures, and this factor was therefore disregarded, in this series.

The results of all observations of peripheral and central pressures are shown in table 1.

It will be seen that the peripheral pressures of normal subjects varied from 102 mm. to 56 mm.; auricular pressures from 61 mm. to 8 mm. The gradients varied

from 76 mm. to 12 mm., with a mean of +41 mm. As a matter of fact, in this group of subjects with apparently normal circulations, all arm-to-heart pressure gradients were 25 mm. or over, except for one case. This was a man of 56, recovering from pneumonia. He was being allowed up in a chair, still had a mild tachycardia at the time of the examination. Whether this case should be considered as having a normal circulation, or whether he should be placed in the group of "subnormal circulation" as found by Starr (12) in post-infectional conditions, is perhaps debatable.

The absolute value of right auricular pressure, in the six cases where the position of the tip of the catheter was determined by lateral x-ray, varied from 61

TABLE 1

Pressures in right auricle and in arm vein, in subjects with normal circulation, and with congestive heart failure

SUBJECT	AGE	DIAGNOSIS	VENOUS PRESSURE, MM. H_2O	
			Arm	Right auricle
C. N.	51	Ca. of sigmoid	75	45
J. Ri.	38	Ca. of bladder	70	8
C. C.	60	Renal tuberculosis	63	38
J. Re.	73	Arteriosclerosis	59	23
F. K.	41	Normal	56	21
P. T.	47	Normal	76	26
G. T.	62	Ca. of liver	102	26
D. P.	56	Pneumonia	73	61
G. P.	43	Lead poisoning	81	49
Average			74	33
L. L.	59	Arteriosclerotic heart	214	216
G. B.	47	Chronic nephritis	128	128
		(later, failure more advanced)	182	184
M. H.	32	Constrictive pericarditis	291	285
A. R.	56	Coronary thrombosis	78	71
W. D.	67	Coronary thrombosis	48	31
J. W.	42	Rheumatic heart	61	40

mm. to 8 mm., with a mean of +37 mm. This is of interest, since most textbooks assume the blood pressure in the right auricle of man to be approximately zero. This value has evidently been taken over from animal data. In the supine position in man, the heart tilts sharply upward,—the more so the deeper the chest,—so that the tip of the right ventricle is well above the right auricle. A zero or negative auricular pressure would be unfavorable for adequate ventricular filling, though of course such filling would be aided in so far as the negative intrathoracic pressure is effective within the heart chambers.

Another point to be mentioned is what *part* of the relatively roomy right auricle the tip of the catheter actually rested in. As measured in our lateral x-rays, in most cases the catheter tip lay about 2.5 cm. from the posterior margin of the

heart shadow, which would be perhaps slightly posterior to the exact middle of the right auricle.

Turning now to the measurements of peripheral venous and auricular pressures in cases of heart disease, we find a striking contrast to the values in normal subjects (table 1). In all four observations on patients in congestive failure, the elevated venous pressures were associated with a sharp decrease or practical disappearance of peripheral-central pressure gradients, auricular and arm pressures being nearly identical. The phenomenon is apparently the same as that described above in animals with right heart failure associated with acute pulmonary edema.

In the three measurements on cardiac patients recovering compensation (table 1), peripheral-central pressure gradients appeared in two to be within limits of normal; in the third, the gradient of only 7 mm. was less than normal.

Discussion. In normal subjects, though the gradients of pressure from arm to heart are somewhat variable, the mean value of +41 mm. of water is similar to that observed between extremity and right auricle in animals.

This gradient must depend largely upon two factors, the size of the venous channels from arm to heart, and the amount of blood flow through them; the arm-to-heart pressure difference being greater, the larger the blood flow and the narrower the venous channels.

It would be expected that under the conditions of heart failure, with dilated peripheral veins and decreased total blood flow, the arm-to-heart pressure differences would decrease; and this is strikingly shown in the actual measurements in congestive failure. There must have been in all cases some excess of peripheral over central pressure, in order for any blood flow to have continued; but in three of the observations this gradient was so small that it was not detectable in the measurements obtained.

Finally, a point of some interest, on which these data offer a very limited amount of information, is the adequacy or inadequacy of various venous pressure reference points that have been suggested as the "zero," or position of the right auricle within the chest. This subject has recently been well presented by Lyons, Kennedy and Burwell (13), and need not be reviewed here.

Figure 4 gives the difference between the actual position of the catheter tip in the right auricle (represented by the horizontal zero line), and the position of the right auricle as it would be estimated, in each of six cases, by the use of various reference points. It will be seen that for these cases, the reference point of Eyster and that of Lyons, Kennedy and Burwell, are closest to the actual position of the right auricle. Other reference points, however, are grouped well together. If, for example, an arbitrary zero level were taken 7 cm. below the angle of Louis, there would be, for these six cases, a good approximation of the actual position of the catheter in the right auricle.

While varying gradients of venous pressure between arm and heart make any peripheral pressure measurement inaccurate as a measure of right auricular pressure, even if the position of the latter is accurately known, it is nonetheless important from the practical standpoint to have some approximately valid

anatomical reference point for venous pressure measurement. With the accumulation of sufficient data, of the type given in figure 4, this could readily be determined.

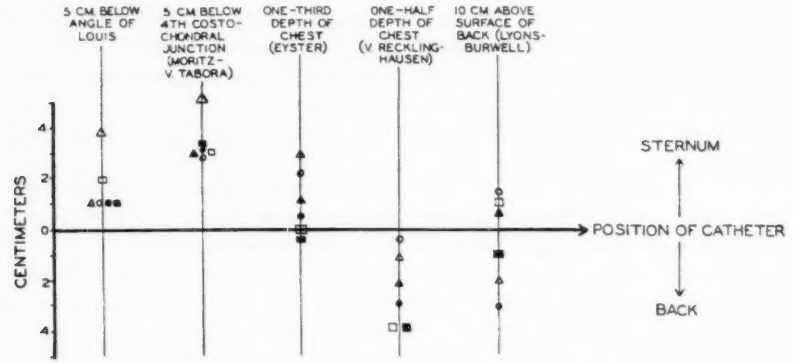

Fig. 4. Relation of actual position of catheter in the right auricle, to the supposed position of the right auricle as estimated by various investigators, in 6 normal human subjects.

SUMMARY

1. In a study of venous pressures in one chimpanzee and a series of dogs, the development of right heart failure associated with acute pulmonary edema was accompanied not only by rise of peripheral and right auricular pressures, but also by a disappearance of the normal pressure gradient between peripheral veins and right heart, the right auricular and peripheral venous pressure levels becoming nearly equal.

2. In nine human subjects with apparently normal circulations, right auricular pressure was recorded directly by means of right heart catheterization. The average gradient from arm to heart was +41 mm. of water. In six subjects absolute pressure levels at the right auricle were determined by locating the position of the catheter by lateral x-ray; the average right auricular pressure (subjects supine) was +37 mm.

3. Three patients in congestive heart failure, with high peripheral venous pressures, showed decrease in peripheral-central pressure gradients, the pressures in arm vein and in right auricle being almost identical.

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THE INTERRELATIONSHIP OF p-AMINOBENZOIC ACID AND INOSITOL

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The discovery of the chromotrichial action of p-aminobenzoic acid by Ansbacher (1) precipitated a controversy as to the true nature of the grey hair factor or factors. Unna et al. (2) and Emerson (3) failed to find any chromotrichial action attributable to p-aminobenzoic acid and believe that pantothenic acid is the only grey hair factor. Martin and Ansbacher (4) noted a chromotrichial action of p-aminobenzoic acid in mice. It seemed obvious that some factor must be at play not recognized by either group of investigators. To bring these various observations into harmony has been the objective of this work.

PROCEDURE AND RESULTS. The basic diet used throughout these experiments consisted of:

	per cent
Vitamin free casein	18.0
Sucrose	67.0
Salts	4.0
Butter fat	9.0
Cod liver oil	2.0

The diet was made up each week, and the vitamin supplements were added as follows:

	mgm. per kilo of diet	*daily dose per rat*
Thiamine hydrochloride	5.0	50 μg.
Riboflavin	10.0	100 μg.
Pyridoxine	5.0	50 μg.
Nicotinic acid	100.0	1 mgm.
Calcium pantothenate	100.0	1 mgm.
Choline chloride	200.0	2 mgm.
Inositol	200.0	2 mgm.
p-Aminobenzoic acid	100.0	1 mgm.

Each experimental group consisted of 30 Rockland strain black rats.

Diet 1. Inositol and pantothenic acid deficiency: This diet contained six factors but was deficient in pantothenic acid and in inositol. On this combined deficiency, the Rockland strain black rat attains a weight of approximately 55 to 65 grams and death occurs within five or six weeks. Spectacled eyes are seen within two weeks. The involvement of the eyes develops to a pan-ophthalmitis. This is bilateral and severe. Purulent exudate, bloody exudate, rupture of

eyeballs and complete blindness characterize the syndrome. It is far more marked than we have noted in a straight pantothenic acid deficiency or in a straight inositol deficiency. There is some alopecia of a very mild variety. Greying or achromotrichia is not a pronounced manifestation. The animals are a brownish grey. The fur has a wet, oily appearance. These animals before death invariably show a peculiar gait; the hind legs are handled awkwardly and do not seem to respond in coördination with the forelegs. Complete paralysis was not seen in any of the thirty animals in this group but fully 90 per cent showed this spasticity.

Diet 2. Inositol deficiency: This diet contained seven of the factors listed and was deficient in inositol. The growth rate of this set is such that after four or five weeks the average weight is 70 to 90 grams. At five weeks the animals show a pattern greying which is markedly similar to that of a pantothenic acid deficiency. Spectacled eyes are common, with pan-ophthalmitis occurring infrequently and not to the marked degree seen in a combined inositol and pantothenic acid deficiency. Again, in this group the locomotor incoördination occurs, but to a lesser degree; and the mild alopecia observed might be called rather a thinning of the hair than real alopecia. The syndrome associated with inositol deficiency is difficult to distinguish from that of a pantothenic acid deficiency.

Diet 3. p-Aminobenzoic acid deficiency: Eighty per cent of the animals were dead within six weeks. The weights of this set reached 50 to 60 grams. The appearance is most unusual: the hair over the body is sleek and black and appears darker than its original color; the hair over the head is sparse, with a mild greying. Crusted, bloody, scaly feet were frequently seen. These animals seem to have no energy; they move sluggishly with hunched backs.

Diet 4. Pantothenic acid deficiency: No deaths occurred in this group during the first eight weeks. The weights attained averaged 70 to 100 grams. A typical pattern greying was seen, accompanied by the usual spectacled eyes. A mild form of alopecia was noted. Pan-ophthalmitis was seen in but two of the thirty rats on this pantothenic acid deficient diet.

Diet 5. This diet contains the eight members of the B complex listed. At six weeks, the weight attained averaged 110 to 180 grams. The animals seemed normal in every respect except that the coat was rather brownish. This diet was supplemented with wheat germ oil, ethyl linoleate and biotin, and found to be complete as evidenced by a failure of biotin or the essential unsaturated fatty acids to increase the growth rate.

Diet 6. This diet contained no inositol and no p-aminobenzoic acid, but did contain the other six factors as listed. These rats do not differ in any way from those on diet 5. The weight, the appearance, in every respect, they are the same. Thus, in the absence of both inositol and p-aminobenzoic acid, the six factors, namely, thiamine, riboflavin, pyridoxine, choline, nicotinic acid and calcium pantothenate are adequate.

Discussion. The results of Unna et al. (2) and Emerson (3) are confirmed in that the six basic factors (thiamine, riboflavin, nicotinic acid, pyridoxine, choline and calcium pantothenate) are adequate for seemingly normal nutrition.

They are not adequate if either p-aminobenzoic acid or inositol is added to the diet. Ansbacher (1) and Martin and Ansbacher (4) had inositol in their basic supplements and thus p-aminobenzoic acid deficiency is noted. Neither Unna et al. (2) nor Emerson (3) had inositol in the basic diet used. It is possible that the explanation lies in a stimulation and/or inhibition of bacterial growth in the intestinal tract and hence the bacterial synthesis of vitamin factors, known or unknown in nature. Both factors, namely, inositol (5) and p-aminobenzoic acid (6, 7), have been demonstrated as growth factors for yeast or bacteria, and p-aminobenzoic acid has been demonstrated to inhibit bacterial growth (8) at certain concentrations. The utilization and consequent destruction of various vitamins by microörganisms has been demonstrated (9, 10). Further, the synthesis of certain factors, particularly biotin (11) and inositol (12) by organisms present in the gastro-intestinal tract has been reported. The stimulation of the growth of microörganisms by one member of the B complex causing an increased synthesis of another member of the B complex by that same organism is not only probable but certain. The analysis we place on the above reported results is that inositol stimulates the growth of organisms which utilize and destroy some member of the B complex, known or unknown, thus precipitating a deficiency of that factor. p-Aminobenzoic acid either through stimulation or inhibition of bacterial growth precipitates an inositol deficiency (4). This seems the logical explanation, in view of the fact that the elimination of both inositol and p-aminobenzoic acid from the diet, feeding only six of the B complex factors results in a state of nutrition which is the equal of that noted if the eight factors are included.

The addition of inositol to a diet may stimulate the growth of organisms which destroy pantothenic acid, whereas the addition of p-aminobenzoic acid may inhibit the growth of these organisms. This explanation can be advanced even if inhibition is not considered, as specific stimulation may cause an overgrowth of one type of organism and the consequent elimination from the gastro-intestinal tract of another. In the preliminary experiments, it is our opinion that proteus vulgaris is absent from the gastro-intestinal tracts of rats fed excessive amounts of p-aminobenzoic acid (5 mgm. daily). Further, we have noted a tendency on the part of the acid forming organisms to overgrow. Thus, we see both an apparent inhibition of proteus and a stimulation of the lactic acid forming organisms. The seeming inhibition of proteus may be actually due to overgrowth on the part of the acid forming organisms.

It is not desired to suggest an alteration of the status of either inositol or p-aminobenzoic acid as a member of the B complex, but to point out that one precipitates a deficiency of the other under our experimental conditions.

The addition of p-aminobenzoic acid to the basic diet made it possible for Martin and Ansbacher (4) to produce an inositol deficiency, where in the absence of p-aminobenzoic acid no inositol deficiency developed (13). Woolley, who originally described this syndrome (14), stated subsequently that in many instances spontaneous cures occurred (15). It is these spontaneous cures which are prevented by the p-aminobenzoic acid. The cures were due to synthesis of

inositol by organisms present in the gastro-intestinal tract (12), and it seems probable that it is this synthesis which is prevented by p-aminobenzoic acid.

CONCLUSIONS

Six B complex factors (thiamine, riboflavin, pyridoxine, choline, nicotinic acid and calcium pantothenate) afford seemingly normal nutrition to the Rockland strain black rat. Addition of inositol precipitates a syndrome prevented by p-aminobenzoic acid. Addition of p-aminobenzoic acid precipitates a syndrome prevented by inositol. Eight B complex factors (the six listed above plus inositol and p-aminobenzoic acid) afford seemingly normal nutrition to these rats.

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INFLUENCE OF ANTERIOR PITUITARY EXTRACT ON PROTEIN AND CARBOHYDRATE METABOLISM

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The anterior pituitary gland is known to act as an anabolizer of protein.

Treatment of rats (7) and dogs (1, 2, 18) with anterior pituitary extracts containing growth hormone results in decrease of N excretion. The N.P.N., Urea N and Amino N of the blood is decreased in dogs, rats and guinea pigs (19, 1, 14). The N.P.N. and its constituents in the tissues are decreased. At the same time the total N content of the tissues increases, thus indicating an increase in protein N (8). Mirsky (13), on the basis of his experiments on depancreatized and on eviscerated dogs, came to the conclusion that the anabolism of protein-following injection of anterior pituitary extracts is due to increased insulin output. Anterior pituitary extract increases protein breakdown. But in presence of the pancreas the latter is stimulated by a concomitant pancreatotropic action of the anterior pituitary extract to increased insulin output. The latter in turn anabolizes protein, thereby not only compensating, but overcompensating the direct protein-catabolizing action of the pituitary extract. The net result in the intact animal, therefore, is protein anabolism or protein "sparing."

The following experiments were performed in order to compare the action of anterior pituitary extract on protein and carbohydrate metabolism. Some parallelism should be expected if the anabolism of protein were an indirect action due to increased insulin output. It was, furthermore, to be examined whether either of the actions on protein and carbohydrate metabolism is dependent on the adrenals or the pancreas. A brief preliminary report on these studies has appeared (15).

METHODS. Adult male white rats were used. Animals were fed Purina dog chow, with lettuce given twice weekly. They had free access to tap water. Adrenalectomized animals received 1 per cent sodium chloride solution instead of tap water. Pancreatectomized animals were fed a liquid diet[2] twice daily by stomach tube. Since other studies, to be reported elsewhere, were performed on them, they were kept in individual metabolism cages and their urines were collected in 24 hour periods.

[1] J. Ewing Mears Fellow in Medicine and Physiology.

[2] *Diet.* The diet consisted of: Starch, 540 grams; Dextrin, 260 grams; Sucrose, 265 grams; Egg albumin, 200 grams; Salt mixture, 40 grams (McCollum-Davis N-185) (5); Cellu flour, 120 grams; Vi-Penta, 10 cc.; Wheat germ oil, 10 cc.; *Mazola oil*, 10 cc.; made up with water to 2000 cc.

The anterior pituitary extract used was Antuitrin G (Parke, Davis & Co.).[3] The extract was injected intraperitoneally.

Blood samples were drawn by heart puncture. No anesthetic was used. Blood was immediatey pipetted into test tubes containing the appropriate protein precipitants without adding any anticoagulant.

Urea N was determined by the method of Rappaport and Glaser (17) with one minor modification—N/100 H_2SO_4 was used as a recipient instead of hypobromite and N/100 NaOH was consequently used for titration, neutral red serving as indicator (16). Two-tenths cubic centimeter of blood was used for every determination.

Blood sugar was determined by Benedict's micromethod, using 0.1 cc. of blood for every determination (5).

Adrenalectomy was performed under ether anesthesia through the usual dorsal incision. Animals were used for experiments 5 to 6 days after operation. During this period they received the laboratory stock diet, but with 1 per cent sodium chloride solution instead of tap water.

Partial pancreatectomy was performed under ether anesthesia in young rats weighing about 80 grams.[4] Animals were used for experiments only several months later after they had reached a weight from 150 gams to 250 grams. During this time they received the laboratory stock diet. After they had reached full weight, they were put in individual metabolism cages and received forced feeding as mentioned above.

All animals were fasted for about 16 hours prior to the experiments.

RESULTS. *Group 1—normal rats.* Twenty adult male rats fasted for about 16 hours received 1 cc. of Antuitrin G intraperitoneally. Blood was drawn before, and 3 and 6 hours after injection. The urea N content of the blood decreased in all experiments, the decrease varying from 10.5 mgm. per cent to 3.5 mgm. per cent (average 7.4 mgm. per cent). The blood sugar decreased in 13 of the 20 animals (65 per cent), in 6 the blood sugar remained constant and in 1 there was a marked rise. Arbitrarily in these and the following experiments changes of blood sugar of less than 10 mgm. per cent were considered as within limits of possible normal fluctuation. Only changes of 10 mgm. per cent or more were considered significant.

There is no parallelism of the degree of decrease of Urea N and of decrease of blood sugar. Thus in rat 67 the Urea N decreased 10.5 mgm. per cent (from 13.6 to 3.1 mgm. per cent). There was no decrease of blood sugar. In rat 68 the Urea N decrease was 10.1 mgm. per cent (from 13.6 mgm. per cent to 3.5 mgm. per cent) and the blood sugar decreased 49.5 mgm. per cent (from 84.5 to 35.0 mgm. per cent).

Group 2—adrenalectomized rats. Twenty-four adrenalectomized male rats

[3] Antuitrin G used in these experiments was generously supplied by Parke, Davis & Co. through the courtesy of Dr. E. A. Sharp.

[4] The author is indebted to Dr. D. J. Ingle for kindly demonstrating the method of partial pancreatectomy. The method employed is in all important points identical with that published since by Harrison and Long (4).

fasted for about 16 hours were studied after intraperitoneal injection of 1 cc. of Antuitrin G in the same way as were the normal animals. Decrease of Urea N varied from 14.7 mgm. per cent to 5.3 mgm. per cent (average 8.2 mgm. per cent). Decrease of blood sugar was found in 13 of 24 animals (54 per cent). Again there appeared to be no correlation of drop of blood sugar to that of Urea N.

As is indicated by the normal range of fasting blood sugars and Urea N values, none of the animals was in a condition of severe adrenal insufficiency. They were adequately maintained through sodium chloride, although some of them lost weight between operation and experiment. Most of the animals died in the days following the experiment, after withdrawal of sodium chloride. Completeness of adrenalectomy was checked by autopsy. However, no search was made for extra adrenal cortical tissue.

Group 3—partially depancreatized rats. Thirteen experiments were performed on 9 rats. Urea N content of the blood decreased in all experiments, varying from 11.9 mgm. per cent to 3.8 mgm. per cent (average decrease 8.4 mgm. per cent). Blood sugar decreased in 6 of 13 experiments (46 per cent). This group is much less uniform than are the two former, inasmuch as the degree of pancreatic insufficiency and consequently the severity of the diabetic condition varied to some extent. Five animals had only occasional mild glycosuria and no glycosuria on the day preceding the experiment. Two of these 5, however, had a moderate fasting hyperglycemia. Three of the 9 rats later received stilbestrol as diabetogenic agent (6). In all 3 stilbestrol aggravated the diabetes considerably. These 3 animals were tested for their reaction to the pituitary extract before and after administration of stilbestrol.

Rat P-1 received a total of 1.9 mgm. Stilbestrol subcutaneously in oily solution over a period of 4 days. No glycosuria was observed before stilbestrol treatment and a glycosuria up to 830 mgm./24 hours in the period after treatment. A single injection of 1 cc. Antuitrin G in the period before treatment resulted in decrease of Urea N of 5.6 mgm. per cent. The blood sugar did not change. The same experiment performed 4 days after stilbestrol treatment (glycosuria on the day preceding the experiment 780 mgm. per cent, on the day of experiment 396 mgm. per cent) produced an increase of blood sugar of 72.9 mgm. per cent. The Urea N decreased 12.9 mgm. per cent.

Rat P-2 received a total of 1.9 mgm. Stilbestrol in oily solution subcutaneously over a period of 4 days. Glycosuria before stilbestrol treatment varied from 20 to 100 mgm. per 24 hours. After treatment glycosuria reached a maximum of 630 mgm./24 hours. A single injection of Antuitrin G in the period before treatment resulted in decrease of Urea N of 4.9 mgm. per cent. The blood sugar increased 12.4 mgm. per cent within 6 hours. Unfortunately no 3 hour sample was taken.

The same experiment 4 days after Stilbestrol treatment produced a decrease of Urea N of 7 mgm. per cent. Blood sugar did not change during the first 3 hours and rose 40 mgm. per cent after 6 hours. Glycosuria on the day preceding the experiment was 630 mgm./24 hours.

Rat P-3 received a total of 7.8 mgm. Stilbestrol in oily solution subcutaneously over a period of 11 days. No glycosuria was observed before stilbestrol treatment. After treatment there was glycosuria up to 470 mgm./24 hours. Injection of 1 cc. of Antuitrin G during the period prior to stilbestrol treatment was followed by a decrease of Urea N of 3.8 mgm. per cent and a decrease of blood sugar of 19 mgm. per cent during the first 3 hours and of 29.6 mgm. per cent during the 6 hour period.

One day after Stilbestrol treatment the experiment was repeated. Urea N now decreased 10.9 mgm. per cent, blood sugar decreased 13 mgm. per cent during the first 3 hours and returned to the fasting level of 92 mgm. per cent after 6 hours. On the day preceding the experiment there was glycosuria of 340 mgm.

TABLE 1

TYPE OF EXPERIMENT	BLOOD SUGAR						UREAN					
	Fasting		3 hours after inj.		Difference		Fasting		6 hours after inj.		Difference	
	Mean	σ*	Mean	σ*		$\sigma_{Diff.}$†	Mean	σ*	Mean	σ*		$\sigma_{Diff.}$†
Normal.....	88.5 (13)‡	12.32	60.0	15.78	28.5	5.19	12.4 (11)	2.48	5.1	2.4	7.3	1.04
Adrenalecto-mized.....	86.8 (13)	10.53	67.0	12.06	19.8	4.44	13.3 (15)	2.99	5.1	2.05	8.2	0.94
Pancreatecto-mized	118.4 (6)	20.14	93.6	11.52	24.8	9.48	13.0 (13)	2.48	4.6	3.58	8.4	1.20

* $\sigma = \sqrt{\frac{\Sigma(\bar{x})^2}{n-1}}$

† $\sigma_{Diff.} = \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}$ (A. B. Dill: Principles of medical statistics, 1937.)

‡ Figures in brackets indicate the number of experiments, from which means have been calculated.

It is apparent that neither the magnitude of the blood Urea N decrease nor the blood sugar response following injection of Antuitrin G is correlated to the severity of the diabetes, the severity being judged by glycosuria or fasting hyperglycemia.

Statistical evaluation. In table 1 statistical evaluation of the foregoing data is given. The means and standard deviations for the blood sugar values have, of course, been computed from the experiments only which showed hypoglycemia following injection of the pituitary extract. It can be readily seen that the drop of blood sugar is significant, since the difference (fasting blood sugar -3 hour value) is more than 5 times $\sigma_{Diff.}$ in the normal rats and more than 4 times $\sigma_{Diff.}$ in the adrenalectomized animals. The pancreatectomized rats show a much greater variation since, as mentioned above, the group included diabetes of

varying severity. Consequently, the difference (fasting blood sugar −3 hr. value) is only 2.3 times $\sigma_{Diff.}$

The decrease of Urea N is uniform in all three groups, the difference (fasting Urea N −6 hour value) is significant, its value being 7 to 8 times $\sigma_{Diff.}$

In table 2 the correlation of changes of blood sugar and of changes of Urea N has been computed. All experiments are included in this table. Since the blood sugar in some experiments did not change, and in some showed a rise, the mean blood sugar decrease of all experiments is considerably less than the mean of the hypoglycemic values as computed in table 1. The correlation coefficient is low. The independence of the blood sugar changes and of the Urea N changes is evident.

DISCUSSION. Anterior pituitary extracts are known to induce nitrogen retention as indicated by increase of total nitrogen in the tissues (8), decrease of N excretion in the urine (1, 2, 7) and decrease of N.P.N., Urea N and Amino N in the blood (1, 14, 15, 19). Decrease of Urea N was used as indicator of the action of the pituitary extract on protein metabolism in the present experiments. Using a micromethod, 0.4 cc. of blood only was needed for duplicate determina-

TABLE 2

	BLOOD SUGAR DECREASE M	BLOOD UREA N DECREASE M	r^*
	mgm. per cent	*mgm. per cent*	
Normal	−21.4	−7.3	0.46
Adrenalectomized	−12.4	−8.2	−0.13
Pancreatectomized	−7.1	−8.4	−0.25

$$^*r = \frac{M(x \cdot y) - (Mx) \cdot (My)}{\sigma_x \cdot \sigma_y}$$

tions of Urea N and additional 0.2 cc. for duplicate blood sugar determinations. It was thus possible to study the blood chemistry in short term experiments of 6 hours.

Mirsky (13) has concluded from his experiments both on depancreatized and on eviscerated dogs that the anterior pituitary gland enhances protein breakdown. This action, however, is masked in the intact animal by a concomitant pancreatotropic action of anterior pituitary extract. This leads to increased insulin output and the insulin in turn acts as anabolizer of protein.

Anterior pituitary extracts have been found to decrease the blood sugar levels of dogs (20) and of rats (4). Weinstein working on dogs in short term experiments used the same extract, Antuitrin G, as did the present author. Harrison and Long treated their rats for two and three days with two types of extracts, one a saline extract, the other an alkaline extract prepared after Burns and Ling.

These findings of a decrease of blood sugar following treatment with anterior pituitary extracts would seem to support Mirsky's views. However, in the experiments reported in this paper a decrease of blood sugar which might be considered indicative of increased insulin output was found in only 65 per cent

of the normal animals treated with anterior pituitary extract whereas the Urea N values were decreased in all animals irrespective of the presence or absence of a drop of blood sugar.

If the protein anabolism were due to an increased insulin output stimulated through a pancreatotropic action of the anterior pituitary extract, blood sugar changes should be roughly parallel to the changes in nitrogen metabolism. The lack of such parallelism makes the above mentioned theory unlikely.

In the experiments of Harrison and Long (4) both alkaline and saline pituitary extracts lowered the blood sugar of fasting rats. The saline extracts also lowered the N.P.N. and reduced the urinary nitrogen excretion whereas the alkaline extracts failed to exert any detectable effect on protein metabolism. This observation would seem to indicate as do our experiments that the actions of anterior pituitary extracts on protein metabolism and on carbohydrate metabolism are not necessarily linked.

Moreover, adrenalectomized animals are known to be more sensitive to insulin. Consequently, if the pituitary extract acted by stimulating insulin output, the effect of the induced hyperinsulinemia should be more marked in adrenalectomized animals. However, the blood sugar drop occurred in about the same percentage of adrenalectomized rats (54 per cent) and normal rats (65 per cent). The difference of 65 per cent normal and 54 per cent adrenalectomized rats showing a drop of blood sugar can hardly be considered significant considering the number of experiments performed (20 normal, 24 adrenalectomized rats). But if anything it would rather tend to show a greater number of normal rats developing hypoglycemia. Also the actual drop of blood sugar was not more marked in adrenalectomized than in normal rats. On the contrary the average blood sugar decrease of the 13 normal rats was 29.2 mgm. per cent as against an average fall of blood sugar of 19.7 mgm. per cent in 13 adrenalectomized rats. The difference of these two values is 9.5 ± 4.3. The significance is doubtful since the value of the difference is only slightly more than $2\ \sigma$ but again, if anything, it would tend to show a lesser degree of hypoglycemia in the adrenalectomized rather than in the normal rats.

In all animals, both normal and adrenalectomized, there was a decrease of Urea N, indicating the action of the anterior pituitary extract on protein metabolism. The decrease of Urea N was present whether or not the blood sugar was lowered. And in those experiments in which a decrease of blood sugar did occur the magnitude of this decrease did not parallel the magnitude of decrease of Urea N. The same holds true also for the third group of experiments, those on partially depancreatized rats. The decrease of Urea N was present whether or not the blood sugar decreased. Moreover, both the changes of blood sugar and those of Urea N seemed to be independent of the severity of the diabetes. It should, however, be noted that none of the rats was very severely diabetic. In 2 of the 13 experiments on pancreatectomized rats the blood sugar increased following the injection of the extract, in one as much as 72.9 mgm. per cent, in the other 9.1 mgm. per cent. However, a rise of blood sugar was also observed in one experiment on normal rats (no. 74) and in one adrenalectomized animal

(no. 58). Gaebler and Galbraith (3) found the same extract, Antuitrin G, severely diabetogenic in completely depancreatized dogs. Of course, we do not wish to imply that an occasional rise of blood sugar in the acute experiments is to be considered as a diabetogenic effect. In three partially depancreatized rats Stilbestrol was given as a diabetogenic agent. Confirming the results of Ingle (6), glycosuria increased considerably. These three rats were tested before and after Stilbestrol administration; in other words, in a stage of mild diabetes and again in a stage of more severe diabetes. These experiments also failed to give any indication that either the decrease of Urea N or the hypoglycemia following injection of Antuitrin G is correlated to the severity of the diabetes.

It is known that the diabetogenic action of certain anterior pituitary extracts is mediated in part at least through the adrenal cortex and that pituitary extracts are not diabetogenous in the absence of the adrenal cortex (11, 12). Young (21) has suggested the hypothesis that the growth hormone acts as such including its protein anabolizing action as long as the pancreas is intact. When the islet response is impaired the same hormone is diabetogenic. It may be of importance in this respect to note that in contradistinction to the diabetogenic effect the protein anabolizing effect is not mediated through the adrenals. As has been shown in this paper, this effect is equally present in intact and in adrenalectomized rats. This result is in accord with that of Harrison and Long (4).

The decrease of blood sugar induced by anterior pituitary extract also is independent of the adrenals. This fact not only raises doubt as to the pancreatic origin of this hypoglycemia, as has been mentioned above; it also makes it difficult to interpret the blood sugar lowering effect and the diabetogenic effect as being two phases of one and the same factor or process.

The fact that both hypoglycemia and decrease of blood Urea N following anterior pituitary extract injection in partially depancreatized rats is independent of the severity of the diabetes also militates against the assumption of a mediation of the anterior pituitary effects through the pancreas. However, since we know nothing of the reactivity of the pancreatic remnants, the experiments on partially depancreatized rats are not conclusive.

SUMMARY AND CONCLUSIONS

1. An anterior pituitary extract containing growth hormone (Antuitrin G) regularly produced a decrease of blood Urea N, indicative of protein anabolism, in the normal fasted rat. In 67 per cent (13 of 20 expts.) there was a concomitant decrease of blood sugar. However, the drop of Urea N was present whether or not the extract produced hypoglycemia. The magnitude of the decrease of blood sugar and blood Urea N were not correlated.

2. In the adrenalectomized fasting rat maintained with sodium chloride, the pituitary extract produced the same decrease of Urea N. The hypoglycemia following injection of Antuitrin G was neither more frequent nor more marked

in the adrenalectomized rat than in the normal. The hypoglycemia was perhaps less marked, but the significance of the difference is doubtful.

3. In the partially pancreatectomized, diabetic rat the decrease of Urea N and the hypoglycemia induced by Antuitrin G did not differ from those in the normal animal. The changes in these blood constituents did not seem to be correlated with the severity of the diabetes in the individual animal.

4. These results would indicate that the enhancing effect of anterior pituitary extracts on protein anabolism is not mediated either through the adrenals or through the pancreas. It also is improbable that the hypoglycemia induced in a number of animals by administration of anterior pituitary extract is due to the increased output of insulin, since adrenalectomized rats would be expected to be more sensitive to an excess of insulin than normal animals.

5. The growth hormone is generally supposed to be the factor responsible for the changes in protein metabolism. The possible bearings of the results enumerated above on the relationship of the growth hormone to the diabetogenic factor of the anterior pituitary gland are briefly discussed.

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5. The growth hormone is generally supposed to be the factor responsible for the changes in protein metabolism. The possible bearings of the results enumerated above on the relationship of the growth hormone to the diabetogenic factor of the anterior pituitary gland are briefly discussed.

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THE INFLUENCE OF DIETHYL-STILBESTROL ON CARBOHYDRATE METABOLISM IN NORMAL AND CASTRATED RATS[1]

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In earlier experiments it was shown that the administration of stilbestrol caused an elevation of carbohydrate levels in the intact rat (1, 2). The naturally occurring estrogens have also been shown to raise carbohydrate levels (2, 3, 4). Further evidence of a relation between sex hormones and carbohydrate metabolism is found in the observations that variations in carbohydrate stores are present in animals of different sexes (5, 6) and after castration (7).

In the experiments to be reported at this time a study has been made of the effect of stilbestrol on castrated rats. In addition, further studies have been made on normal animals.

Thirty-one castrated and 26 normal rats received subcutaneous injection of 100 gamma of diethyl-stilbestrol[2] daily for periods of 5, 10 or 20 days. Castrations were performed at least three weeks before treatment was begun. Twelve castrate and 14 normal uninjected animals were used as controls. All animals were fasted for 36 hours before killing and urine was collected in sulphuric acid during the last 24 hours of the fast. As in our earlier studies (1) determinations were made for blood sugar, liver- and muscle-glycogen, and urinary nitrogen. The gonads, adrenals and pituitaries were weighed and together with the mammary glands were prepared for microscopic examination.

The blood sugar levels were variable as is indicated by their standard deviation (table 1). However, the figures for these probably are within the normal range for rats fasted 36 hours, except in the case of the animals treated for 20 days. In the latter instances the blood sugar levels of the females (table 2) showed an appreciable elevation. It is our experience that the blood sugar level will rise for 5 or 6 hours after an injection of stilbestrol. The same phenomenon has been noted by Zunz and Labarre (8) after the administration of estradiol benzoate. However, the acute effect was eliminated in the present experiments since the rats received their last injection on the day before they were sacrificed.

Deuel, Gulick, Gruenwald and Cutler (6) found that intact female rats which had been given specific quantities of glucose showed higher blood sugar levels

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[2] Diethyl-stilbestrol was supplied to us through the kindness of Dr. Richard Johnson of the Frederick Stearns Company and Dr. J. A. Morrell of E. R. Squibb & Sons.

than males treated in a similar fashion. In the present experiments only the females which had been treated for 20 days showed an appreciable difference in blood sugar values. This rise was less striking in the male animals which had

TABLE 1

Averages of normal and castrated rats treated with 100 gamma stilbestrol daily

NUMBER OF ANIMALS	TREATMENT	WEIGHT AT END OF 36-HOUR FAST	BLOOD SUGAR	GLYCOGEN		URINARY N LAST 24 HOURS	WEIGHT OF PITUITARY	WEIGHT OF ADRENALS (2)	WEIGHT OF OVARIES (2)	WEIGHT OF TESTES (2)
				Liver	Muscle					
		gm.	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm./ gm. body wt.*	*gm./100 gm. body wt.*	*gm./100 gm. body wt.*	*gm./100 gm. body wt.*	*gm./100 gm. body wt.*
14	Normal	167	69 ± 4*	83	323	0.826	0.0047	0.0249	0.0400 (8)†	1.359 (6)†
12	Normal castrated	217	71 ± 8*	115	342	0.868	0.0052	0.0189		
8	Normal 5 day stilbestrol	163	72 ± 8*	239	293	0.748	0.0077	0.0357	0.0503 (3)†	1.270 (5)†
9	Castrated 5 day stilbestrol	206	75 ± 8*	285	331	0.694	0.0068	0.0304		
10	Normal 10 day stilbestrol	149	74 ± 8*	331	311	0.774	0.0083	0.0346	0.0537 (6)†	1.122 (4)†
9	Castrated 10 day stilbestrol	192	73 ± 7*	323	355	0.812	0.0084	0.0410		
8	Normal 20 day stilbestrol	131	84 ± 6*	409	260	1.042	0.0110	0.0550	0.0595 (8)†	
13	Castrated 20 day stilbestrol	188	83 ± 11*	594	354	0.960	0.0109	0.0407		

*Standard deviation.

†Numbers in parentheses are number of animals.

TABLE 2

Averages of normal and castrated male and female rats treated with 100 gamma stilbestrol daily

NUMBER OF ANIMALS	TREATMENT	SEX	WEIGHT AT END OF 36-HOUR FAST	BLOOD SUGAR	GLYCOGEN LIVER	GLYCOGEN MUSCLE	URINARY N LAST 24 HOURS	WEIGHT OF ADRENALS (2)	PITUITARY
			gm.	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm./gm. body wt.*	*gm./100 gm. body wt.*	*gm./100 gm. body wt.*
6	Normal	M	178	68	83	316	0.765	0.0208	0.0039
8	Normal	F	160	70	83	329	0.872	0.0283	0.0053
8	Castrated	M	228	72	124	335	0.878	0.0191	0.0050
4	Castrated	F	194	68	99	356	0.844	0.0191	0.0056
5	Normal 5 day stilbestrol	M	159	76	220	237	0.787	0.0304	0.0057
3	Normal 5 day stilbestrol	F	161	67	282	383	0.686	0.0461	0.0090
3	Castrated 5 day stilbestrol	M	225	79	330	299	0.649	0.0241	0.0050
6	Castrated 5 day stilbestrol	F	196	73	261	347	0.717	0.0340	0.0070
4	Normal 10 day stilbestrol	M	153	76	296	319	0.749	0.0307	0.0064
6	Normal 10 day stilbestrol	F	147	73	354	305	0.791	0.0374	0.0097
3	Castrated 10 day stilbestrol	M	193	77	367	236	0.888	0.0404	0.0085
6	Castrated 10 day stilbestrol	F	191	71	302	421	0.774	0.0415	0.0083
8	Normal 20 day stilbestrol	F	131	84	409	260	1.042	0.0549	0.0110
4	Castrated 20 day stilbestrol	M	205	77	638	387	0.839	0.0388	0.0088
9	Castrated 20 day stilbestrol	F	181	86	577	340	1.018	0.0408	0.0119

received similar treatment. However, a larger number of animals must be studied before this material can be considered as statistically valid.

In animals treated with stilbestrol stored carbohydrates in the form of liver

glycogen were affected most markedly while muscle glycogen showed little variation from the normal. Liver glycogen values for normal fasted rats were a little higher than had been expected (see table 1) and probably reflect the habit of coprophagy which is observed in many rats. In each group of treated animals the liver glycogen was elevated in direct relation to the length of treatment. Although this was a consistent observation within each group, an occasional animal failed to respond to the treatment. These have been included in the averages. The observations of Deuel et al. (6) and Grayman, Nelson and Mirsky (5) on the occurrence of higher levels of liver glycogen in male rats which had received glucose after an initial fast would suggest that we might have expected a sex difference in the glycogen levels of our rats. Although our animals received no carbohydrate after the initiation of fasting, a tendency toward a sex difference in glycogen levels was manifested.

In general, values for liver glycogen were higher in castrated than in normal rats (table 1) and higher in male than in female castrates (table 2). In this regard Zain (7) observed that the administration of Progynon B to hyperthyroid and hyperthyroid-castrated rats resulted in the occurrence of higher values for liver glycogen in the castrates and higher values for males than for females. Our results on normal castrates are similar to Zain's findings on hyperthyroid-castrates. The augmented effect of stilbestrol in castrates may be associated with a lower basal metabolic rate in such animals. Experiments designed to determine this possibility are in progress.

Urine for nitrogen determinations was collected during the last 24 hours of the 36 hour fast. This method is open to some error but it offers an indication of the N.P.N. excretion. As would be expected, the nitrogen values were variable. Since rats which have been treated with stilbestrol lose weight, a rise in the excretion of nitrogen might be expected and it is hazardous to interpret increased N.P.N. levels as being related to such shifts as may have been observed in carbohydrate levels. Nevertheless, females treated for 20 days did show an appreciable rise in urinary nitrogen (table 2) and this may indicate the source of the increased levels of liver glycogen.

Stilbestrol, as is true of the naturally occurring estrogens, has definite effects on many of the endocrine glands. The adrenals and pituitaries were increased in size and, in general, were larger in females than in males. They showed, as a rule, a progressive hypertrophy with continued treatment. No consistent difference was observed in the responses of these glands in normal and castrated rats. The pituitaries of treated animals showed a progressive degranulation of the chromophilic cells and an increase in chromophobic cells.

The ovaries reached a maximum size in rats injected for 5 days and showed large corpora lutea and small follicles. The entire male reproductive system was damaged and the seminiferous tubules and interstitial cells of the testes showed marked atrophy.

The mammary glands of all animals which received stilbestrol showed various degrees of stimulation. In general, the effect was similar to that seen in rats treated with estrone or estradiol (Nelson, 9).

It is evident from these experiments that the action of stilbestrol in increasing carbohydrate levels is not through the gonads. In fact, the liver glycogen values, in general, are greater in castrates than in normals. Further studies are in progress in an attempt to determine whether stilbestrol acts through the adrenals or the pituitary or whether its effect on carbohydrate levels is independent of these glands.

CONCLUSIONS

1. Stilbestrol tends to increase blood sugar levels after 20 days' treatment in fasting normal or castrated rats.

2. Liver glycogen values are increased after 5 days of treatment, and considerably more after 20 days. This elevation, in general, is greater in males than in females, greater in castrates than in normals. Muscle glycogen is not altered after the administration of stilbestrol.

3. Urinary nitrogen values are variable but they are somewhat increased after 20 days' treatment, and may indicate the occurrence of gluconeogenesis from protein.

4. The adrenals and pituitaries are increased in size. In general, they are larger in females than in males.

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OBSERVATIONS ON THE PHYSIOLOGICAL REACTIONS OF THE DUCTUS ARTERIOSUS

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In a previous paper (Kennedy and Clark, 1941), we have pointed out that the ductus arteriosus is a blood vessel having special characteristics which distinguish it from other large vessels, both in its structure and in its physiological reactions. The normal structural characteristics and the changes following normal closure were presented, and it was pointed out that patency of the ductus arteriosus, one of the common forms of congenital heart disease, may be due to the failure of a physiological mechanism rather than to an embryological malformation. The present paper is concerned with physiological reactions of the ductus.

We have already presented evidence to show that the closure of the ductus arteriosus is a process having two distinct phases. The first is an immediate reaction taking place a few minutes after birth and is essentially a contraction of the muscular wall of the ductus (fig. 1). In some species there has been described a flap-like valve (Hamilton, Woodbury and Woods, 1937) which may aid in closure by obstructing the flow of blood from the aorta into the ductus, but this latter mechanism does not appear to be a factor in the guinea-pig fetus, the animal forming the basis of our study (Harmon and Herbertson, 1938). The second phase of closure is much slower than the first and involves histological closure of the lumen and the replacement of the muscular elements in the wall by fibrous connective tissue resulting in the conversion of the ductus into the ligamentum arteriosum.

The ductus seems to have the capacity to close in response to a variety of stimuli, and in this respect its reaction is quite different from that of the aorta and pulmonary artery. We observed that following the onset of breathing in normal guinea pigs closure of the ductus arteriosus occurred within 4 to 10 minutes. After a few experiments it became apparent that closure of the ductus followed artificial inflation of the lungs with air as well as normal breathing. This suggested that the reaction of closure occurred as a reflex response to definite stimuli, and in an effort to throw light on this conception we entered on the present series of experiments.

MATERIALS AND METHOD. We have used more than 90 pregnant guinea pigs and made observations on upwards of 175 fetuses. By using the technique described previously, which involves delivering the fetuses by operation beneath

the surface of a warm saline bath and leaving the placental circulation and cord vessels intact, prolonged observations can be made on each fetus and the various reactions of the ductus can be studied. The spinal cords of the mother guinea pigs were sectioned in the lower thoracic region under ether anesthesia in preparation for all experiments except a few and where any of the latter were used in the experiments given as examples, special mention is made of it in the protocols. Most of the fetuses used were near term. In some animals the day of fertiliza-

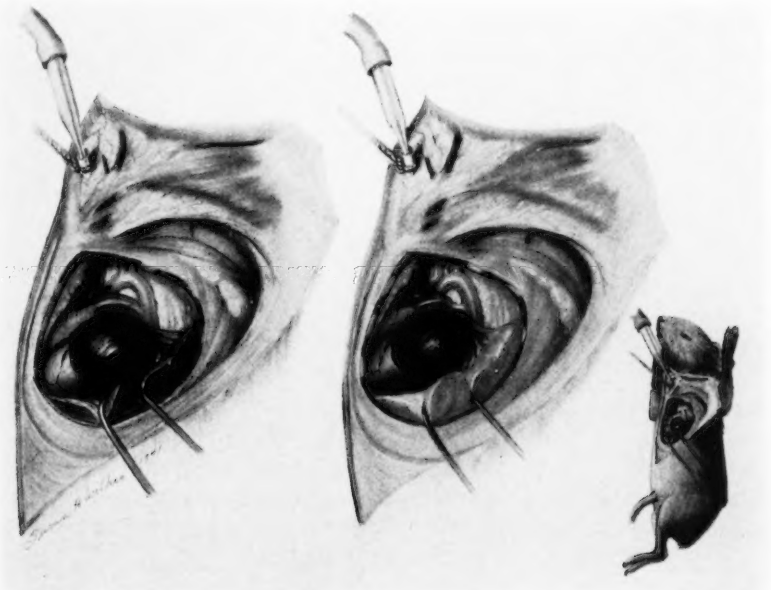

Fig. 1. Drawing showing the appearance of the ductus arteriosus of fetal guinea pig open and closed. In the figure the arch of the aorta is shown in the upper part of the opening of the chest, with the pulmonary artery just below it, apparently continuous (by way of the ductus) with the descending aorta (cf. fig. 2). The ductus in the larger right hand figure closed following artificial inflation of the lungs with air. The lighter color of the lung margin (being retracted below the heart) following inflation is apparent. The smaller figure to the right gives the appearance of the fetus with opening in chest wall. In each figure the tracheal cannula is shown.

tion and length of pregnancy were accurately known. All the fetuses used were weighed and the crown-rump lengths measured. These were compared with the weights and lengths appearing in published data on normal guinea pigs. According to Draper (1920) and Ibsen (1928) guinea pig fetuses at term have an average weight of 80 to 85 grams and are about 10 cm. in length. The individual weights vary with the size of the litter from 70 grams with 6 fetuses to 110 grams with 1 fetus (Ibsen, 1928). The gestation period for guinea pigs has an average duration of 68 days (Ibsen, 1928).

RESULTS. In fetuses near term it was found that the ductus will close following several different types of stimuli (fig. 2) and it will subsequently open again if the stimulus producing the closure is discontinued. This sequence of closing and opening can be repeated at will. For example, intermittent rhythmic inflation of the lungs with puffs of air through a tracheal cannula at a rate of about 50 per minute (to simulate breathing) caused the ductus to close 7 times consecutively in the same animal, each closure being followed by opening, as shown by the following protocol. Protocol 1. Weight of fetus 93 grams; length 11.4 cm. (crown-rump), selected from a group of such experiments.

Fetus removed from uterus 2:08 p.m. Chest opened and ductus arteriosus observed to be open. 2:11 cannula inserted into trachea.

Ductus open. Times at which successive inflations were begun	Period of inflation required for closure
1. 2:14	2 min., 30 sec.
2. 2:23½	1 min.
3. 2:36½	3 min., 30 sec.
4. 2:47	2 min., 30 sec.
5. 2:56	2 min., 30 sec.
6. 3:05	3 min.
7. 3:14½	2 min., 50 sec.

The ductus arteriosus was allowed to open following each closure.

In lively guinea-pig fetuses near term, this type of inflation of the lungs with air was almost invariably followed by closure of the ductus. It was therefore used as a method for producing closure and various experiments were designed to interrupt the mechanism operating in the hope of determining the underlying causes of closure of the ductus. With the possibility that closure following pulmonary inflation was dependent upon a neurological pathway a series of experiments was performed in which portions of the nervous system were destroyed with the idea of interrupting a reflex mechanism.

Experimental search for reflex paths. Subsequent to each of the following procedures the ductus was successfully closed by intermittent inflation of the lungs through a tracheal cannula:

1. Tissue dissected off anterior surface of ductus (including left vagus and phrenic nerves).
2. Bilateral section of vagus nerves.
3. *a*. Bilateral removal of stellate ganglia.
   b. Bilateral removal of stellate ganglia and section of both vagus nerves.
4. *a*. Bilateral ligation of carotid arteries below bifurcation.
   b. Bilateral removal of carotid arteries including their bifurcation.
   c. Bilateral removal of carotid arteries including their bifurcation plus bilateral section of vagus nerves.
5. Ligature pulled very tightly around all vessels, nerves and other structures of mediastinum, cephalic to aortic arch (except trachea).
6. Lesions of spinal cord alone.
   a. Cross section of cord at level of 3rd cervical; 6 thoracic; 9th thoracic segments. (Some destruction of cord on each side of section.)
   b. Destruction of large portions of spinal cord; from 2nd cervical to 2nd thoracic segment; 3rd cervical to caudal end; 1st thoracic to 11th thoracic segment.

7. Lesions of spinal cord *plus* other structures.
   a. Spinal cord destroyed (T1 to T10) and both vagus nerves cut.
   b. Spinal cord destroyed (C2 to caudal end), all mediastinal structures cephalic to aortic arch (except trachea) tied with ligature and both vagus nerves cut.
   c. Entire spinal cord and medulla destroyed, both stellate ganglia removed, both vagus nerves cut.

Note: The procedures listed were carried out on more than one fetus. For example, closure of the ductus following inflation was observed in eight animals after bilateral vagus

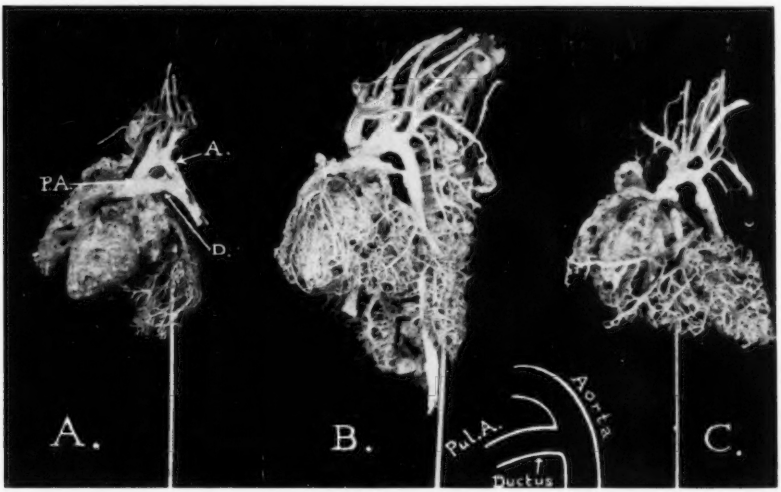

Fig. 2. Photograph of casts of lumina of vessels from injected guinea pig fetuses. The small diagram shows the relation of the great vessels. (A) The ductus arteriosus open. (B) The ductus constricted. (It had closed following inflation of lungs with oxygen but had partly relaxed before the injection fluid was introduced.) (C) The ductus completely closed. This fetus was born normally and injected after several hours of normal breathing. The casts were made by injecting a liquid solution of bakelite[1] into the great vessels of the thorax. After hardening of the bakelite, the tissues were macerated in strong sodium hydroxide solution and washed away leaving a cast of the cavities of vessels injected. (The vertebrae remain in B.) Due to the pressure necessary for injection the recently closed ductus (B) was found to contain a fine thread of the injection mass, which represents the lumen and indicates by its form that the ductus constricted over its entire length. The casts shrink slightly on hardening which accounts for certain irregularities. Magnification ×2.

nerve section; in four after bilateral carotid removal; in eight after destruction of the spinal cord; in three after bilateral removal of the stellate ganglion, etc.

The above procedures, in which all known neurological pathways between central nervous system and region of the ductus were interrupted, failed to prevent closure of the ductus following inflation of the lungs with air. The only possible neurological mechanism which might have remained operative would be

[1] Vinylite.

a reflex dependent upon local neurons but there is not supporting evidence for the presence of such a local reflex.

The problem of the mechanism of closure was also approached by a different type of experiment. Various structures were stimulated with an electric current of low voltage (60 cycle sine-wave) and the effect on the ductus noted. We have stimulated in this way the left vagus nerve, right vagus nerve, left cervical sympathetic, the left phrenic nerve, the left stellate ganglion, the left splanchnic nerve without causing any noticeable change in the ductus.

It appears from the above experiments that a nerve pathway or a neuromuscular reflex is not essential for closure of the ductus. It also appears that the ductus will not close following stimulation of certain nerves which are anatomically closely associated with it.

It may be appropriate to point out here that in experiments of this sort there are variables present to disturb the accuracy of the results; such, for example, as interference with the circulation of the fetus, or that of the uterus or placenta, etc. For this reason one positive experiment in which the ductus closed is worth more than one in which no closure occurred. At times when experiments similar to some of those described above as resulting in closure were performed the ductus remained open. Those in which closure occurred are more significant because they show that the destructive lesion did not interrupt the mechanism of closure under the conditions of the experiment.

As previously stated the ductus was observed to close following several types of stimuli other than artificial inflation of the lungs with air. The various procedures employed and their effects upon the ductus can best be shown under the following headings with protocols of a few representative experiments.

1. *Normal breathing.* Protocol 2. Weight of fetus 88 grams; length 11.2 cm. (crown-rump).

Fetus delivered by operation 4:29. Umbilical cord intact. Spontaneous, vigorous breathing began at 4:31. Opening of chest begun 4:35 30″. Ductus observed to be closed at 4:35 45″.

2. *Mechanical or electrical stimulation of ductus.* Any dissection in the vicinity of the ductus which causes tugging on its wall, or gentle pinching of the ductus with small tissue forceps, or electrical stimulation of its wall is followed by closure. This reaction differs from the preceding one in that closure following a local stimulus occurs more promptly, usually within 15 to 30 seconds, and in that it can occur even a short time after death of the fetus. Similar stimulation of the great arteries of the thorax is not followed by a reaction similar to that of the ductus but the umbilical vessels constrict promptly at the site of mechanical stimulation.

3. *Artificial inflation of the lungs with oxygen and nitrogen.* Protocol 3. The ductus was allowed to open following each closure.

Closure of the ductus with these three methods (normal breathing, direct mechanical or electrical stimulation, and artificial inflation of the lungs) has been observed many times. While artificial inflation failed at times, there was usually observed some factor which might have affected the normal response, such as failure of either the fetal or maternal circulation, length of experiment, temperature of bath, size and age of fetus, etc.

Other observations listed below (sections 4 to 7) have not been repeated a sufficient num-

DUCTUS OPEN. INFLATION OF LUNGS WITH:	NUMBER MINUTES INFLATION	STATE OF DUCTUS ARTERIOSUS AT END OF INFLATION
Fetus 1, weight 128 grams; length 12.3 cm.		
Oxygen	6	Closed
Nitrogen	10	Open
Oxygen	4.5	Closed
Nitrogen	7	Open
Oxygen	1.75	Closed
Fetus 2, weight 117 grams; length 11.8 cm.		
Nitrogen	15	Open
Oxygen	6	Closed
Nitrogen	8	Open
Oxygen	4.5	Closed
Nitrogen	5	Open

ber of times to test fully their reliability in closing the ductus but we have observed several valid experiments of each kind.

4. *Injection of adrenalin.* a. Protocol 4. Pregnant female guinea pig decerebrated under ether. Fetus near term (weight and length not recorded). Chest opened. Ductus open. One cubic centimeter of 1/10,000 solution of adrenalin injected slowly into the heart. Ductus closed 3 minutes 10 seconds after injection begun.

b. Protocol 5. Weight of fetus 84 grams; length 11 cm.; 0.5 cc. adrenalin chloride 1/10,000 injected into subcutaneous tissue of abdomen. Ductus closed 2 minutes 20 seconds after injection.

5. *Mechanical stimulation of carotid sinus.* a. Protocol 6. Weight of fetus 72 grams; length 10.2 cm. Left carotid sinus massaged with blunt end of probe. Ductus which had been open, closed after 2 minutes' massage.

b. Protocol 7. Weight of fetus 71 grams; length 10.2 cm. Right carotid sinus massaged with blunt end of probe. Ductus which had been open, closed after 4 minutes 20 seconds' massage.

6. *Hemorrhage.* Protocol 8. Weight of fetus 70 grams; length 10 cm. Ductus had remained open and under observation for 25 minutes. Left external jugular vein was cut for purpose of causing hemorrhage which was short and brisk. Ductus closed 6 minutes after beginning of hemorrhage.

7. *Unexplained.* On several occasions we observed closure of the ductus and were not able to relate it definitely to any of the above factors. For example, in about half a dozen experiments after the tracheal cannula was inserted the ductus closed; or after destruction of the spinal cord by thrusting a pipe cleaner into the vertebral canal; or after opening the chest wall with an associated small hemorrhage the ductus closed; or after the position of the fetus was changed suddenly, and occasionally after the mother struggled, the ductus closed. On the whole these unexplained closures were infrequent but they suggest that there may be factors operating to cause closure which we do not understand and do not at present recognize. It may well be that closure which followed some other procedure should be grouped here, or that some of these experiments belong under another heading.

8. *Intravenous injection of oxygen.* From the evidence that has been presented it is difficult to arrive at a conception of the cause of closure of the ductus which will fit all the facts observed. The most promising observations from the standpoint of a mechanism used at birth are those concerning the different reactions following inflation of the lungs with air, oxygen and nitrogen. According to our experiments, inflation of the lungs with

pure nitrogen does not result in closure of the ductus and it seems that oxygen is an important constituent of the gas mixture when closure follows inflation of the lungs. If oxygenation of the fetal blood causes closure, then oxygen given to the fetus by any route resulting in oxygenation of the blood should have the same effect. In order to test this hypothesis the following experiment was devised. With the usual technique the ductus was visualized and by means of a small needle and a tuberculin syringe pure oxygen was slowly injected into the umbilical vein in a series of tiny bubbles. This procedure was promptly followed by closure of the ductus in four animals. In one other, whose heart was beating irregularly a partial constriction of the ductus occurred. In two others the technique of injection failed and the experiment could not be completed. In no one of these animals were the lungs inflated before the oxygen was injected.

Protocol 9. Weight of fetus 69 grams; length 11.3 cm. Oxygen injected into umbilical vein very slowly with small needle. The ductus which had been open, closed after 3 minutes 15 seconds, a total of 0.3 cc. of oxygen was injected.

Note: The irritability of the umbilical vessels to needle puncture can be abolished by first painting the outside of the cord with formalin (10 per cent), then injecting a few tenths of a cubic centimeter of formalin into the mucous connective tissue of the cord around the vessels.

Discussion. We have established that the ductus arteriosus is a structure which can actively close in response to certain stimuli. It reponds to local mechanical stimulation much the same as certain other hollow muscular structures by contracting. We do not believe that local mechanical stimulation has an essential rôle in its closure under physiological conditions. Neither does a neurological mechanism appear essential to closure following artificial inflation of the lungs. Our findings are at variance with those of Barcroft, Kennedy and Mason (1938) with respect to the reaction of the ductus following stimulation of the vagus nerve, but we believe that the present observations have been adequately controlled.

Of the stimuli causing closure of the ductus which we have explored, it seems likely that under physiological conditions, breathing is the most important. The actual filling of the lungs by just any gas is not sufficient. From our experiments it appears that oxygen is a necessary component of the gas mixture since inflation of the lungs with pure nitrogen will not cause closure. Oxygen by vein will also cause closure without the necessity of accompanying inflation of the lungs. It is quite possible that many or all of the unexplained closures (see sec. 7) could be due to an increased oxygenation of the fetal blood in response to painful stimulation, struggling of the mother or fetus, hemorrhage, etc. There are other possible sources of stimulation which we have not yet explored fully, such as various natural humoral substances, CO_2, drugs, etc.

Such an influence as that of oxygen on the ductus may have something in common with the findings of Figge (1934) who demonstrated a definite effect on the metamorphosis of the aortic arches and gills in larval forms of the salamander by variations in oxygen tension of their environment.

If this seemingly important relationship of oxygen to the mechanism of closure of the ductus is true, it offers a practical indication for treatment of new-born infants, especially those which have difficulty in the oxygenation of their blood.

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TRAINING AND ITS EFFECTS ON MAN AT REST AND AT WORK

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In exhausting activity, such as in competitive sports and in war, the vigor and extent of exercise are circumscribed by the limits of the physiological functions involved. We know that at a given age and under fixed conditions, the heart rate cannot be pushed beyond a certain value. At any given time the capacity for supplying oxygen to the tissues is strictly limited. The individual will work anaerobically until a tolerable limit of oxygen debt and concentration of lactic acid is reached. Severe prolonged work may be limited by the stores of carbohydrate.

Yet it is a common observation that exercise repeatedly carried out leads to an improved performance. In the runner this amounts to running a greater distance at the same pace, or covering the same distance more quickly, or covering the same distance at the same rate with less fatigue. As soon as improvement in performance can be demonstrated, the process of training may be said to have begun. The rate of improvement depends on the individual's initial state and on how rigorous a regime he follows. Any regime systematically followed will have its most striking results following a few weeks of training; after the first rapid gains, hard diligent work is required if a continued improvement is to be secured. Such training is essential for success in amateur or professional sport. The less seriously one takes his sport, the less impressive is the second phase of improvement: the average young man can soon acquire the ability to run a mile in six minutes but he must work hard and long to run it in five minutes.

The means by which training results in an increased capacity for work is not completely understood. Experimental work done on animals and their musculature, and on intact humans, has yielded some significant advances, but some of the evidence is conflicting. An excellent review of work on training was made by Steinhaus (1933), and later Dill (1936) reviewed various aspects of muscular exercise. More recent summaries of the literature related to the problem are those of Hellebrandt (1940), Steinhaus (1941) and the closely related studies of muscular contraction as reviewed by Sacks (1941). Extensive discussions are found in Bainbridge (1931) and Schneider (1939). Many of the studies on training have compared two selected populations, one, a group of trained men and the other, a control group. This type of study yields less useful data than one in which individuals are studied before and during training.

Two years ago simultaneous studies of training were begun in the Fatigue Laboratory and in the Department of Physiology of the University of Indiana.

The subjects consisted chiefly of non-athletic students. The plans for laboratory performance tests and observations were similar but separate paths were followed in order to obtain independent judgments as to the outcome of the experiments. Some of the Indiana results have been published by Robinson and Harmon (1941), and the review in their paper of recent literature is so adequate that it need not be repeated here. It is now clear that the Indiana students showed greater improvement and reached higher levels of performance than did the Harvard students. These differences probably depend on a number of factors, some of them psychological. The students at Indiana were working their way through college and were paid for their time in these tests. Those at Harvard were fulfilling the requirement that all Freshmen take part in some physical activity. They too were paid but not all of them were dependent on this source of income. While both groups had track work three times weekly there is no comparative record of the amount of training received nor of the degree to which individuals pushed themselves during training. The Indiana student, in the words of Doctor Robinson, "were given about as much running as they could take but this was considerably short of what is expected of a veteran runner. The amount of running done by a beginner is limited by shin-splints and arch trouble. These may occur even in some veterans if too much work is attempted."[1]

The observations made in our laboratory consisted of bimonthly studies of the men at rest and biweekly studies of work performance. We measured basal metabolic rate, analyzed alveolar air, recorded breathing curves and vital capacity, obtained resting pulse and blood pressure values. Venous blood drawn during each bimonthly test was analyzed for O_2 capacity, alkaline reserve, plasma protein and chloride. A study was made of the formed elements of the blood and hemoglobin concentration. At the same intervals, the men collected three 24-hour urines for study of possible effects of training on excretion of chloride, creatine and creatinine.[2]

The progress of the subjects was followed biweekly by means of work experiments on our motor-driven treadmill. The subject walked eight minutes at 3.5 m.p.h. on an 8.6 per cent grade, and then immediately ran on the same or a higher grade at 7.0 m.p.h. for five minutes if possible. Otherwise he stopped when exhausted. At the end of the run the subject jumped to the side or straddled the belt, keeping the mouthpiece in place. As soon as the belt stopped a stool was placed on it. The subject sat on the stool during a 15-minute recovery period. Oxygen consumption was measured for the last part of the walk, for each minute of the run and for the recovery period. A continuous heart rate record was made of the entire experiment. Capillary blood was taken after the

[1] Personal communication.

[2] Nearly all of our subjects ate at the dining hall provided for Freshmen and hence had the same choice of food. There was no control exercised over their diet except that over a six-week period some time during the six months each man took 60 grams of gelatin daily. There was no evidence that the gelatin influenced performance nor the training curve, that is, the rate at which performance improved.

run for the determination of sugar and lactate. Since the treadmill grade was adjusted up and down during the series of experiments, the subjects were not exhausted at the end of five minutes in some instances. The experiments reported here, however, are those in which the subjects became exhausted within five minutes. These series were at approximately the same intervals as the basal tests.

RESULTS. *Basal state.* The mean results of the experiments on fasting and resting subjects are shown in table 1. The first figures represent the initial

TABLE 1

Summary of the means of data on the basal state of fourteen subjects in training for middle-distance running over a period of six months

	CONTROL	TRAINING PERIOD		
		After two months	After four months	After six months
Height, *cm.*	176.1	176.1	176.1	176.1
Weight, *kgm.*	69.6	70.8	72.0	71.8
Heart rate	66.8	62.8	63.2	61.8
Blood pressure, *mm. Hg.*	114/65	113/65	111/63	113/67
Respiratory rate	15.1	14.6	14.1	14.3
Respiratory vol., *l./min. NTP*	6.23	5.73	5.60	5.69
Vital capacity, *l.*	3.79	3.67	3.70	3.63
Basal metabolism, *Cal./m.²/hr.*	41.5	40.6	40.8	40.9
Alveolar pCO_2, *mm. Hg.*	41.5	40.8	40.6	41.3
Alveolar pO_2, *mm. Hg.*	100.3	104.4	100.8	98.1
O_2 Capacity, *vols. %*	19.83	20.24	20.14	20.18
Alkali reserve*	47.8	46.9	47.6	46.6
Plasma chloride, *m.Eq./l.*	103.6	104.8	106.0	105.8
Plasma nitrogen, *gm./l.*	10.3	10.7	10.7	10.8
Hemoglobin, *gm./100 ml.*	14.8	15.1	15.0	15.2
Red cells, *millions/mm.³*	4.62	4.82	4.72	4.73
White cells, *per mm.³*	5960	6120	5780	6090
Hematocrit, *%*	44.1	44.7	45.2	46.0
Urine chloride, *m.Eq./day*	186	210	181	201
Urine total creatinine, *gm./day*	1.85	1.92	1.84	1.81

* Defined as the CO_2 combining capacity of oxygenated blood, measured at 37°C. and a pCO_2 of 40 mm. Hg.

values before training began. The next three were obtained after approximately two, four, and six months of training.

Most of the measures in rest, which have been previously thought to be related to the degree of training, appear to remain relatively constant. For example, the alkaline reserve and the alveolar CO_2 tension remain practically level, and if a tendency must be noted, it is for a decrease, rather than an increase, as found in numerous previous studies. On the other hand, there is a decrease in the resting pulse rate of five beats per minute, which fits nicely into the generally accepted picture of training. The gain in weight, amounting to

slightly more than 2 kgm., may be related in whole or in part to the training regime.

Blood pressure, respiratory rate, vital capacity and the basal metabolism were not significantly altered by training. Oxygen capacity of venous blood rose by about 2 per cent, an insignificant change. The increase in concentration of the plasma chloride was at the outside less than 3 per cent, but the change was so consistent that the increases by the fourth and sixth months of training were statistically reliable.

The remaining data on hemoglobin, the formed elements of the blood and the excretion of chloride, creatine and creatinine were more variable in the subjects from test to test, and showed no significant relation to the regime of training.

Grade-walking. In the walk, which was always at the same grade and rate, experienced subjects reach a steady state within three or four minutes of starting, carrying on aerobically without a mounting oxygen debt. The oxygen requirement for this grade of work showed the greatest decline within two months, with a further slight decrease at the end of six months of training (table 2). This is reflected in an increased net efficiency at this rate of work of about one-tenth.

TABLE 2

The mean changes in the efficiency of grade walking

	O_2 PER MINUTE		NET EFFICIENCY		HEART RATE	
	l./min.	Δ%	Per cent	Δ%	Beats/min.	Δ%
Control	1.91		15.3		151	
After 2 months	1.81	−5.2	16.6	+8.5	145	−4.0
After 4 months	1.81	−5.2	16.8	+9.8	145	−4.0
After 6 months	1.78	−6.8	16.9	+10.4	146	−3.3

These two functions are not exactly mirrored because the measurement of oxygen requirement has not been adjusted by subtracting the basal O_2 requirement nor has it been related to body weight. These points are taken into account in measuring efficiency. The increased efficiency of grade walking was accompanied by a decreased heart rate of about 4 per cent. The mean values of R.Q. for the four series were 0.94, 0.93, 0.92, 0.93 respectively, reflecting an extraordinary constancy in the proportion of carbohydrate utilized.

The improved performance in the walk probably depends on better skill and coördination. It is not likely that the training program had much to do with this improvement for even skilled runners may be clumsy and inefficient in their first walk on the treadmill.

Maximal work. The experiments calling for the exhaustion of the subject within five minutes necessitated an increase in grade as the training progressed. The mean grade was increased from 9.1 per cent in the control series to 10.9 in the second, to 13.3 in the third, and to 13.4 per cent in the last series. Observations made on one of the men after he had attained a high capacity are shown in table 3. The mean duration of run for these same series was 3.22, 3.87, 3.17 and

3.44 minutes respectively. Thus, in the last test the run was 7 per cent longer, while the grade was almost one-half greater than in the first test. When the body weight is taken into account, the duration and rate of work yield the total physical work done.

It is of great importance to recognize that the principal increase in work output was accomplished by the increase in rate of work, the duration of work not varying greatly. Under these conditions gains in rate of work performance must decline approaching an asymptote, however rigorous the training. On the other hand, if the rate of work output is kept constant, there may be an enormous increase in the quantity of work that can be done. Thus, with the rate constant, Karpovich and Pestrecov (1941) had a subject who was exhausted after 12 minutes work at the beginning of their tests, but who was able to work for 5 hours, 16 minutes several weeks later. This represents a gain of over 2500 per cent in total work output.

TABLE 3

Typical data on the performance of one man (J. Y.) in exhausting work

		HEART RATE IN RECOVERY
Work		
Treadmill speed	(11.3 km.p.h.)	At 20 sec., 187 beats/min.
Treadmill grade	(15.8%)	At 40 sec., 178 beats/min.
Duration of run	(3.63 min.)	At 60 sec., 171 beats/min.
Physical work	(7000 kgm.-m.)	At 90 sec., 158 beats/min.
Maximal oxygen transport	(3.53 l./min.)	At 2 min., 147 beats/min.
Maximal heart rate	(197 beats/min.)	At 3 min., 132 beats/min.
		At 4 min., 124 beats/min.
Recovery		At 5 min., 123 beats/min.
Net O_2 debt (15 minutes)	(8.63 l.)	At 10 min., 112 beats/min.
Maximal lactate	(144 mgm. %)	At 15 min., 113 beats/min.
Maximal blood sugar	(163 mgm. %)	

To translate the accomplishment of our subjects into more familiar units, we may say that our average man, while running up hill at seven miles per hour could raise his body 180 feet in 3.22 minutes at the beginning, and 284 feet in 3.44 minutes six months later.

Exhausting experiments are characterized by a mounting oxygen debt, indicating that the body processes are working beyond a point where equilibrium can be attained, and much of the work is carried on anaerobically. The mean increase of about 60 per cent in work done through the training period was in part due to a greater use of the anaerobic mechanisms for energy transformation (table 4). This is indicated both by the increased tolerance for lactate of as much as 18 per cent, which is paralleled by a smaller increase of 5 or 6 per cent in the oxygen debt as judged by the amount repaid in the first 15 minutes of recovery. There was also an increase of about 6 or 7 per cent in the transport of oxygen to the tissues during work, which represents a clear-cut gain in the amount of work which could be carried on aerobically. The amount of oxygen

debt measured during the first 15 minutes of recovery from work of this nature represents a proportion of the total debt that may vary from one-half to two-thirds. The index to anaerobic work so obtained is not precise but even making the most unfavorable assumptions it is clear that only a small fraction of the increased work output can have been accomplished by increased anaerobic energy transformation: it must be attributed to improved efficiency in running.

It is interesting to note that the concentration of lactate reached increased about 10 per cent in the second series relative to unit oxygen debt as measured for fifteen minutes. The ratio stayed virtually constant for the remainder of the experiment. The figures were 20.4, 22.5, 22.4 and 22.6 mgm. per cent lactate per liter of oxygen debt. The constancy of the figure leads one to believe that this change was real, whatever its basis.

An analysis of the heart rate data in recovery after maximal work showed that not only was the mean maximal heart rate at the end of the run approximately

TABLE 4

The mean changes in the capacity for exhausting work during training

	WORK DONE TO EXHAUSTION		MAXIMAL LACTATE		MAXIMAL BLOOD SUGAR	
	Kgm.-m.	Δ %	Mgm. %	Δ %	Mgm. %	Δ %
Control	3786		114		127	
After 2 months	5593	+47.7	131	+14.9	142	+11.8
After 4 months	5573	+47.2	135	+18.4	134	+5.5
After 6 months	6046	+59.6	134	+17.5	134	+5.5
	WORK PER LITER O_2 DEBT		MAXIMAL O_2 PER MINUTE		O_2 DEBT FOR 15 MINUTES	
	Kgm.-m.	Δ %	l.	Δ %	l.	Δ %
Control	484		3.45		7.82	
After 2 months	691	+42.7	3.64	+5.5	8.09	+3.4
After 4 months	671	+38.7	3.69	+7.0	8.30	+6.2
After 6 months	737	+52.2	3.69	+7.0	8.20	+4.9

constant but neither did the regime of training materially alter the course of the recovery heart rate. The data are charted in figure 1. The curves appear to be exponential, and can be fitted reasonably well from the first to the fifteenth minute of recovery. The general form of the curve can be expressed, using the exponential function, as $y = ae^{-bx} + c$ where y = heart rate and x = the time elapsed. The curves have been calculated but do not yield any more information than can be obtained from the chart shown. The important thing we wanted to know was whether the heart rate declined more rapidly or reached a lower level as a result of training. Obviously, the curves under the stated conditions are virtually unaffected by training.

Discussion. In the exhausting grade of work we found that the capacity for aerobic energy transformation had been increased, as evidenced by the increase in maximal oxygen intake. This probably depends on an increased cardiac output, on improved circulation to the working muscles, and on more

favorable conditions for exchange of gases between the capillaries and muscle cells.

The increased level of lactate noted in our subjects may be looked upon as one of the most significant effects of training. We have been accustomed to say that the level of lactate at the end of work is a measure of the extent to which a man has pushed himself. It has been recognized that at best this is a rough approximation. In high altitudes Edwards (1936) has shown that the

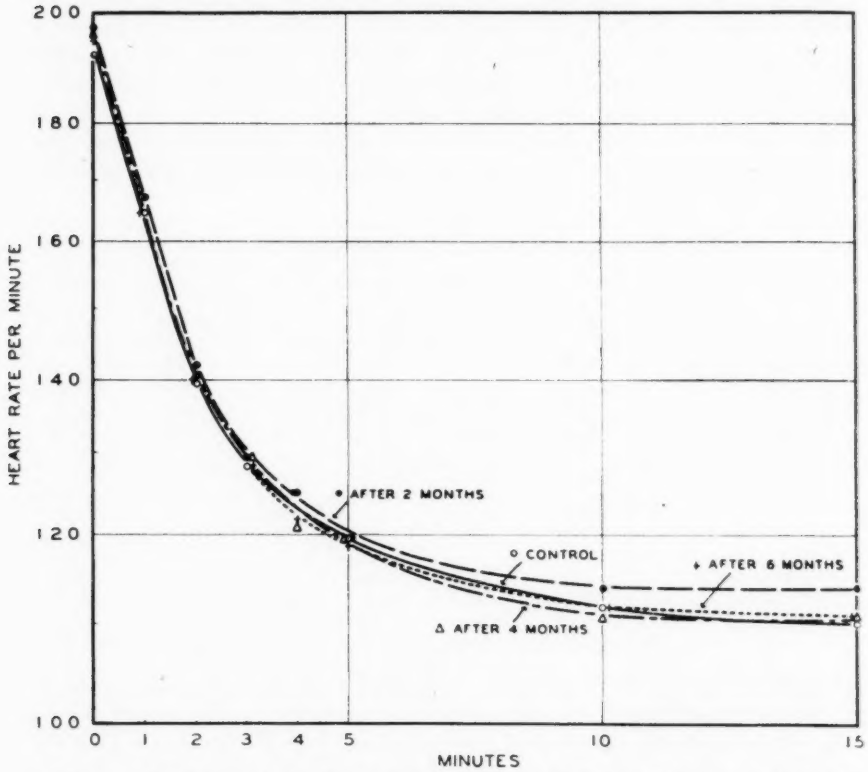

Fig. 1. Training and the heart rate decrement following exhausting work on the treadmill.

capacity of an individual to accumulate lactate in exhausting work falls off as the degree of anoxia, or the altitude, increases. Robinson (1938) has shown that old men, even though healthy, cannot accumulate much lactate: the capacity for anaerobic work falls off with increasing age. On the other hand, the highest lactates we have seen occur in athletic young men. The ordinary young man will stop work with a lactate of 100 mgm. per cent while first-class athletes will continue until their lactate level has reached 150, 175 or even 200 mgm. per cent as has been shown by Robinson and Harmon (1941).

We know that in high altitudes despite the inability to reach sustained high levels of performance in climbing, the individual may have unimpaired muscle strength. Furthermore we have seen excellent performances in strength tests in men past middle age who were incapable of reaching a high level of lactate in work that taxed the capacity for supplying oxygen. These observations suggest that the capacity to accumulate lactate runs parallel and may in fact furnish an excellent index to cardiovascular fitness.

Increases in oxygen intake and in oxygen debt account only in part for the increase during training in the rate at which external work can be done. The useful work output increases in relation to the oxygen requirement.

One of our negative findings is interesting in the light of current indices of physical fitness. Many of these use the decline of pulse rate after performance of a fixed task as one of a battery of tests of physical condition of a subject. We find that a regime of training, which certainly increases physical fitness, does not alter the decline of heart rate following exercise to complete exhaustion within an approximately constant time limit of three to four minutes. A study of the decrement in heart rate after exercise of fixed intensity and duration would certainly show a more rapid pulse recovery during any effective period of training. Our data do not negate such findings nor do they run counter to the assumption that individual differences in performance can be related to decline of pulse rate after moderate exercise. They do seem to show that when complete exhaustion is reached in a given time, the rate of work varying, the pulse recovery curve remains unaffected by training. This is in harmony with the findings of Robinson and Harmon (unpublished data—personal communication).

SUMMARY

A group of fourteen subjects followed a training regime for middle-distance running over a period of six months. The men were studied before and during this period at rest and while doing two grades of work on a motor-driven treadmill.

The training regime was accompanied by a slight increase in weight, a decrease in resting pulse rate of five beats per minute, a slight decline in the respiratory rate and volume and a slight increase in plasma chloride.

No significant differences were found in alveolar CO_2, alkaline reserve, metabolic rate, hemoglobin or the formed elements of the blood, all being measured in the resting state.

An increase in efficiency of grade walking was observed.

In exhausting work there was an increased capacity for supplying oxygen to tissues and greater utilization of anaerobic energy reserves. The work increment unaccounted for by these alterations presumably results from a more economical organization of bodily functions.

The increased capacity for accumulating lactic acid that is developed during training, and the notably high lactate levels attained by first class athletes points to this determination as a useful index to cardiovascular fitness.

We are indebted to Dr. Clark W. Heath for hematological studies reported here and to Mr. Frank Consolazio for invaluable technical assistance.

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THE REACTION AND NEUTRALIZING ABILITY OF THE CONTENTS OF THE PYLORIC ANTRUM AND FIRST PART OF THE DUODENUM IN NORMAL DOGS FOLLOWING AN EWALD MEAL[1]

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Acid has been accorded a leading rôle in both the pathogenesis (2, 3, 9, 10, 11) and the maintenance (3, 6, 9, 10, 11, 12) of peptic ulcer. This study was undertaken primarily for the purpose of learning more about the acidity of the contents of the pyloric antrum and the first part of the duodenum in normal animals. It is hoped that the knowledge gained in this and subsequent investigations will shed some light on the pathologic physiology of peptic ulcer. We hope also to learn whether gastric and duodenal acidities vary independently or follow a parallel course. Such knowledge should assist in evaluating current diagnostic and therapeutic measures which are directed almost exclusively toward measurement and modification of gastric acidity in spite of the fact that at least 85 per cent of the ulcers encountered clinically are in the first part of the duodenum (3, 4, 7, 8, 13).

The observations of duodenal acidity will also extend our knowledge regarding the availability of acid in the intestinal contents for the regulation of gastro-intestinal functions such as gastric and pyloric motility and pancreatic and gastric secretion.

METHOD. Dogs averaging about 20 kgm. in weight were prepared with cannulated gastric and duodenal fistulas as described in previous reports from this laboratory (16, 17). No observations were made for 3 to 4 weeks following operation. All the animals were trained to stand quietly in a muslin hammock-support before any experiments were attempted. The basal routine diet between the experimental periods consisted of daily feedings of raw beef for 5 to 6 days followed by "Purina" dog chow for 1 to 2 days.

[1] Portion of thesis submitted by Dr. Berk to the Faculty of the Graduate School of Medicine of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Medical Science (D.Sc. (Med.)) for graduate work in internal medicine.

[2] Ross V. Patterson Fellow in Gastroenterology.

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After a fasting period of 24 to 36 hours, sampling tubes were introduced through the gastric and duodenal fistulas consisting of 2 soft rubber tubes perforated for a distance of 1 inch from their tips, which were joined together by a thread $\frac{3}{4}$ inch long (fig. 1). The tubes were so placed that the thread came to lie within the pylorus, thereby permitting specimens to be collected from areas 1 inch long on each side of the pylorus. In addition, a small soft rubber instillation tube perforated near its tip was introduced through the gastric fistula (fig. 1). A diagrammatic schema of the final arrangement is shown in figure 2.

After a rest interval of $\frac{1}{2}$ hour, samples in the fasting state were taken simultaneously from the gastric and duodenal segments at 10 minute intervals for another $\frac{1}{2}$ hour. At the completion of this period the dogs were fed an Ewald meal consisting of 2 pieces of stale bread or toast and

Fig. 1

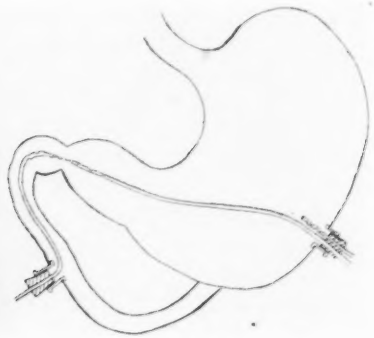

Fig. 2

Fig. 1. Sampling and instillation tubes used in cannulated fistulas.

Fig. 2. Diagram of stomach and duodenum showing the approximate location of the cannulated fistulas and the manner of arranging the sampling and instillation tubes.

250 cc. of tap water at room temperature. The water was introduced through the gastric instillation tube because of the refusal of the dogs to drink it. Beginning 10 minutes after the start of the meal, samples were simultaneously removed from the gastric and duodenal segments at 10 minute intervals for $2\frac{1}{2}$ hours and at $\frac{1}{2}$ hour intervals for another hour. In a few experiments samples were removed at other times in addition to those stated.

The pH of each sample was determined electrometrically through the use of a Leeds-Northrup pH indicator, which uses a glass electrode, and the titratable free acid and total acidity were measured, using dimethylaminoazobenzene (Toepfer's reagent) and phenolphthalein as the respective indicators. On each duodenal specimen, in addition, there was determined what was called the excess neutralizing ability. This indicated the amount of hydrochloric acid (N/10) which could be introduced per 100 cc.

of duodenal contents before the reaction for free acid with Toepfer's reagent became positive. In expressing the results the 4 specimens collected in the fasting state were averaged to obtain a single fasting value.

Twenty-seven acceptable experiments were performed on 6 dogs. All experiments which were done on animals who were later found to have been ill or who vomited and those in which there was uncertainty as to the duration of the fasting state or the position of the tubes were discarded. Of the acceptable experiments, 20 were complete in that they were carried out for the standardized period, and 7 were incomplete in that they were carried out for periods shorter than the arbitrarily selected standard. In all, 2726 different observations were made consisting of 801 determinations of pH, 786 measurements of free acid, 764 measurements of total acidity and 375 measurements of duodenal excess neutralizing ability.

Results. *pH* (fig. 3). There was a consistent difference in pH of samples collected simultaneously from just above and below the pylorus. In both the fasting and post-meal phases, this difference averaged about 3 pH units. The pH values of the contents of the first part of the duodenum displayed a wider range than did the contents of the pyloric antrum. Both, however, failed to follow any regular type of curve, tending rather to fall on a flat although irregular line.

Free acid (fig. 4). The average free acid found in the stomach in the fasting state is probably high, especially for dogs. The foreign body effect of the tubes may have contributed to the production of the high acidity so we do not regard it as especially significant. Following an initial decrease in free acid immediately after the meal, due presumably to dilution effect, there was a progressive increase which tended to stabilize at the peak level at the termination of the period of observation. The increase in titratable gastric free acid was in striking contrast to the absence of any corresponding decrease in gastric pH (fig. 3).

With our technic the contents of the first part of the duodenum characteristically showed an absence of free acid. Out of 410 determinations of free acid in both the fasting and post-meal phases only 11 were positive for free acid (2 fasting and 9 post-meal) and the highest value obtained was 16 clinical units. Our method of preparation of the sample for titration purposes consisted of adding 10 cc. of distilled water to 1 cc. of the strained contents. The pH readings were made on the unfiltered, undiluted specimens as obtained from the sampling tube. The pH at our free acid (Toepfer's reagent) end point averaged 3.36 with a range of 2.88 to 3.72 (1). It will be seen in figure 3 that in the post-meal phase alone 56 of the 342 determinations of pH in the unfiltered, undiluted specimens were 3.5 or below. All of these would be expected to show a positive reaction for free acid on the basis of the pH at our end point with Toepfer's reagent. Yet, after filtration and dilution only 9 or 2.6 per cent yielded positive reactions for free acid. We were able to demonstrate that this error was due primarily to the effect of dilution (1). The average in-

crease in pH effected by dilution was 0.6 unit. Even after qualifying our figures to allow for this error, it may be said that the immediate admixture

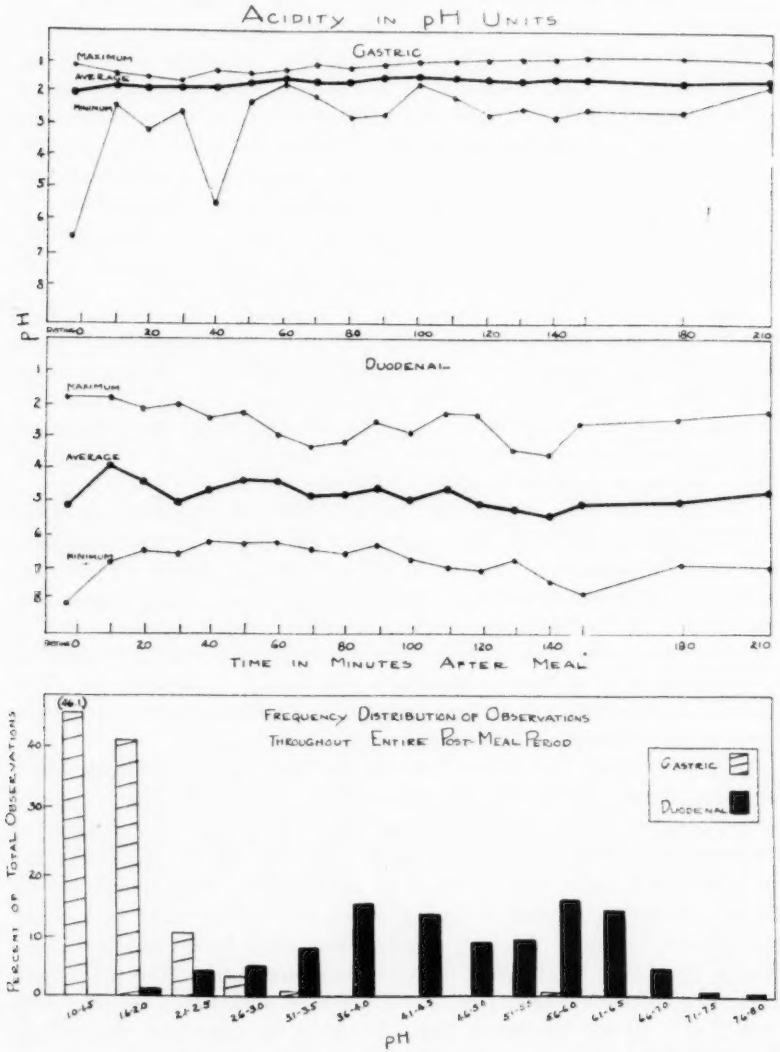

Fig. 3. Acidity in pH units of samples collected simultaneously from just above and just below the pylorus.

of the gastric chyme and the contents of the first part of the duodenum in normal dogs following an Ewald meal will result in complete neutralization of the free acid in 80 to 85 per cent of the samples.

Total acidity (fig. 5). Essentially the same remarks apply to total acidity within the stomach as were made in regard to free acid. A rough

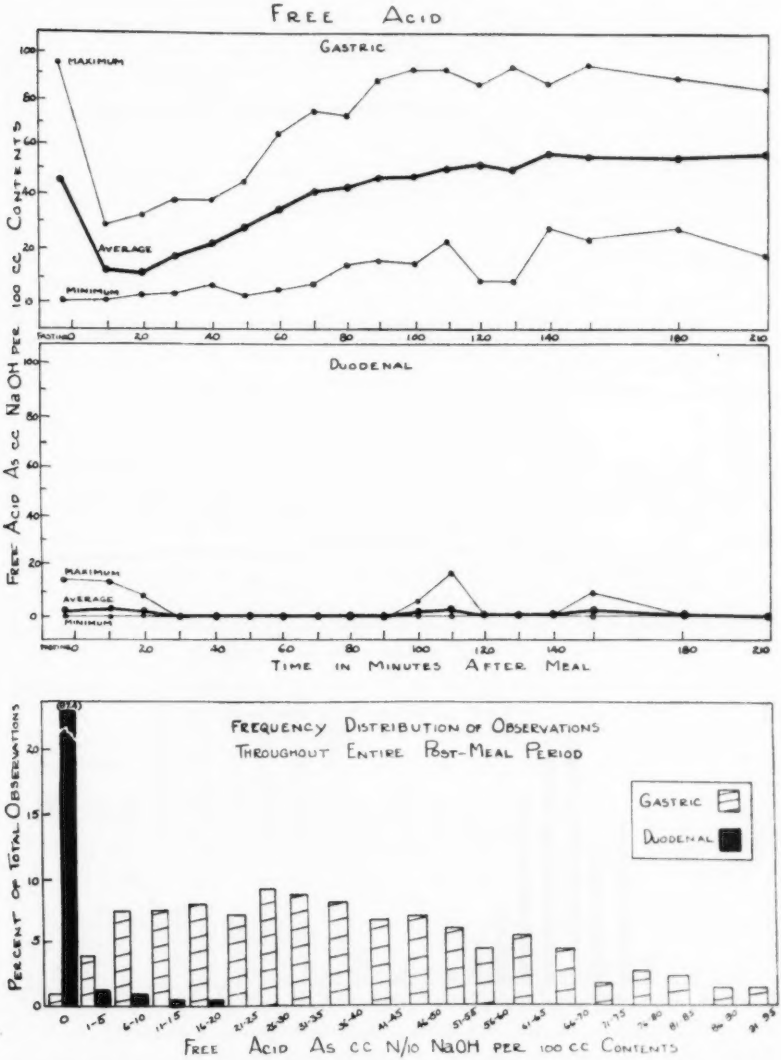

Fig. 4. Free acid as determined on samples collected simultaneously from just above and just below the pylorus.

curve of total acidity can be made out in the duodenal bulb which has a similar configuration to, although it is widely separated from, that seen in the stomach. In both the pyloric antrum and the first part of the

duodenum the increase in total acidity is again in contrast with the lack of any corresponding decrease in pH in either region (fig. 3).

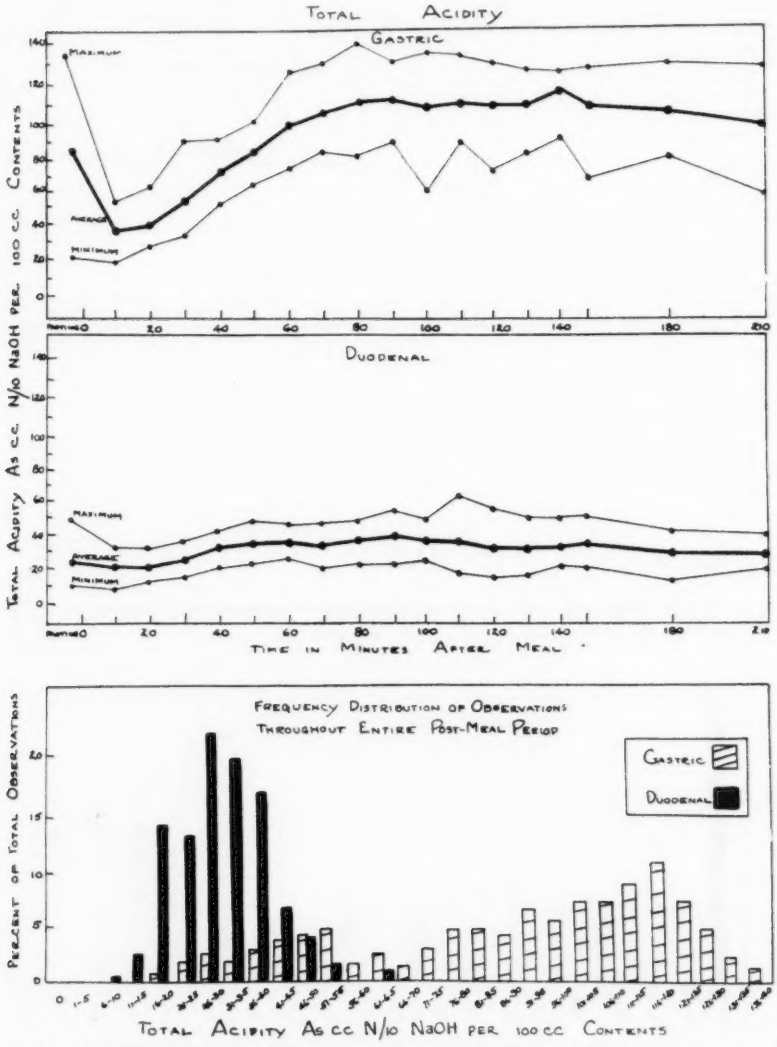

Fig. 5. Total acidity of samples collected simultaneously from just above and just below the pylorus.

Excess neutralizing ability of the duodenal contents (fig. 6). It was noted above (fig. 4) that when gastric chyme and duodenal contents were admixed in the duodenal bulb there occurred almost immediate neutral-

ization of the free acid in the vast majority of post-meal specimens. In order to determine how well the contents of the first part of the duodenum were equipped to neutralize the acid received from the stomach, its excess neutralizing ability was measured. This was done by adding N/10 hydrochloric acid to 1 cc. of the strained, admixed duodenal contents diluted with 10 cc. of distilled water to which 4 drops of Toepfer's reagent was added. The end point was the same as that used for the titration of free acid.

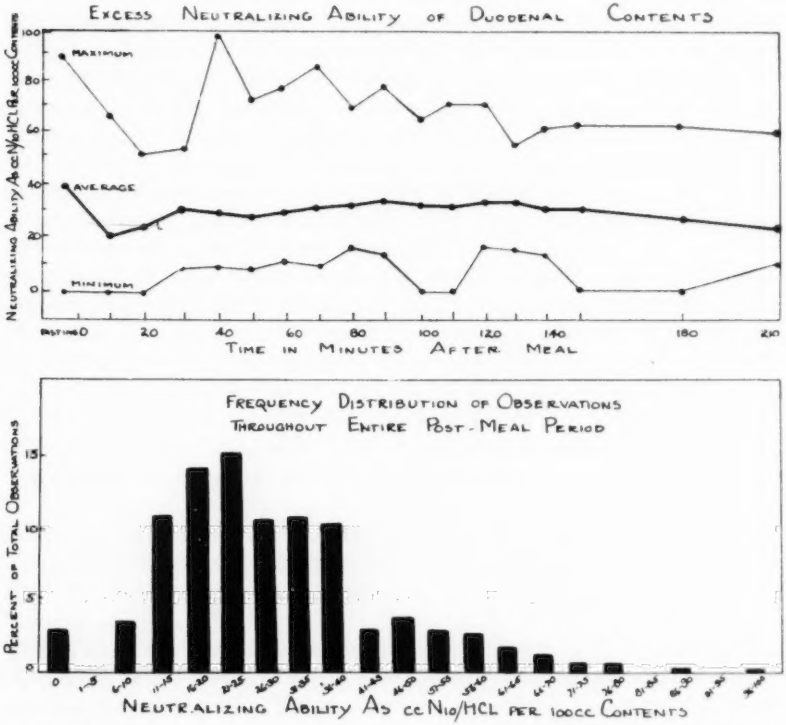

Fig. 6. Excess neutralizing ability of the contents of the first part of the duodenum.

The curve representing the excess neutralizing ability closely parallels that of the duodenal acidity (fig. 5). It is this excess neutralizing ability of the contents of the first part of the duodenum that accounts for the phenomenon of a rise in acid content without a proportional decrease in pH. The error due to dilution which was operative in causing false negative free acid readings in some duodenal samples is likewise active in this determination. It was demonstrated, however, that when the initial pH of the unaltered sample was above 3.5 the effect of dilution on the hydrogen

ion concentration was slight (1). Since the average pH in the duodenal bulb during the post-meal phase was about 4.5 (fig. 3), the altering effect of dilution would only influence a comparatively small percentage of the observations. The corrected values, undoubtedly, would tend to depress the curve to some extent but the excessive neutralizing ability of this part of the duodenum would still be easily apparent.

Discussion. In a previous study one of us (15) has collected data similar to those reported here but following a full meal of raw lean beef instead of the Ewald meal. In the previous study no fasting samples were taken and samples taken within 10 minutes after feeding were not included but this slight difference does not alter the general character of the results. A comparison can, therefore, be made to show the relation of the type and quantity of food to the acidity of the gastric and intestinal contents.

As might be expected, the acidity as measured in pH units of the gastric contents was much higher after the poorly buffered Ewald meal, most of the samples being more acid than pH 2.0 with the peak of the distribution curve falling between pH 1.0 and pH 1.5. In contrast, after the meat meal most of the samples were less acid than pH 2.0 with the peak of the distribution curve near pH 2.6. Samples collected from the duodenum after the Ewald meal were scattered over a wider pH range and showed less tendency to concentrate around a characteristic value than those collected after the meat meal. Distribution curves constructed from the two sets of data both have peaks near pH 4.0 but the curve obtained after the Ewald meal has a second, higher peak, near pH 6.0, a value attained in only two samples following the meat meal. In general the acidity of the intestinal contents was definitely less after the Ewald meal than after meat.

From this comparison the conclusion can be drawn that the acidity of the duodenal contents in the normal dog is largely determined by the type of food undergoing digestion and bears little relation to the acidity (in pH units) of the gastric contents.

Both this and the previous study mentioned above show that in the pyloric antrum as well as in the first part of the duodenum acid is more or less quantitatively buffered as rapidly as it is produced. As a consequence, the pH tends to remain stable in the stomach and to vary in the duodenum independently of the titratable acidity. The first part of the duodenum, in particular, is equipped with a remarkable neutralizing, buffering and diluting capacity considerably in excess of its physiological needs with respect to the free acid of the gastric chyme. Similar conclusions were reached by Imes (5) and by Stevens (14) using a different approach in a study of the reaction of the duodenal contents in parts other than the first part alone.

The consistent difference in pH and titratable acidity of the samples collected simultaneously from just above and just below the pylorus is

so striking as to indicate the existence of a special mechanism whereby the gastric contents are partially neutralized the moment they enter the duodenum. As suggested earlier by one of us (15), such a mechanism may be found in the "receptive relaxation" of the duodenum. Inhibition affecting chiefly the first portion of the duodenum may cause an accumulation of duodenal contents in the vicinity of the pylorus at the moment of exit of the gastric contents, and thus facilitate the quick dilution and partial neutralization of the chyme.

These observations also have a bearing on the question of the occurrence in the intestine of acidities adequate to serve as a stimulus for the regulation of gastro-intestinal functions such as gastric tone and peristalsis, pyloric tonus, and pancreatic secretion. Available evidence indicates that free acid (pH 3.0 or below) in the intestine is needed to affect gastric functions significantly (18, 19) and a pH of 4.0 or below to cause more than minimal stimulation of the pancreas (20). Although acidities below pH 3.0 occurred in our experiments, they were infrequent and were not, in our opinion, present with sufficient regularity or of sufficient duration to account alone for the regulation of gastric emptying. On the other hand acidities of pH 4.0 or below were found in 32 per cent of the duodenal post-meal samples and, therefore, may have been a significant factor in stimulating the flow of pancreatic juice.

SUMMARY AND CONCLUSIONS

1. There is a consistent difference in acidity as indicated by pH measurements of samples collected simultaneously from just above and just below the pylorus in normal dogs fed an Ewald meal.

2. Free acid is usually but not constantly absent in the contents of the first part of the duodenum.

3. Acid that is produced in the stomach is more or less quantitatively buffered in the pyloric antrum and as it enters the duodenum it is quickly diluted and partially neutralized.

4. The contents of the first part of the duodenum in the normal dog display a neutralizing, buffering and diluting capacity apparently in excess of the physiological needs.

5. Although titratable total acidities follow roughly a parallel course in the antrum and duodenal bulb, pH values in both regions vary independently of these and of each other. Likewise, variations in titratable free acid in the stomach are not regularly accompanied by corresponding changes in duodenal pH. Consequently, no measure of gastric acidity can be used as a reliable index of the effective acidity (hydrogen ion concentration) of the duodenal contents in the normal dog.

6. Following a meal consisting chiefly of carbohydrate, the acidity of the intestinal contents is rarely sufficient to affect gastric motility significantly but may be a factor in stimulating pancreatic secretion.

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THE METABOLISM OF FRUCTOSE BY THE EVISCERATED RAT[1]

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Corkill and Nelson (1939) found by analysis of samples of blood and muscle that all the fructose injected at a constant rate for four hours into the surviving, decapitated and eviscerated cat could be accounted for at the end of this time if it was assumed that the sugar was uniformly distributed throughout the blood and muscles and that these composed respectively one-sixteenth and one-half of the total weight of the preparation. This seemed convincing evidence that fructose was not acted on by this preparation, from the circulatory system of which all abdominal organs including the kidneys had been removed. This result, however, was opposed to the findings of McGuigan (1908), Steinberg (1927), Griesbach (1929) and Bornstein and Volker (1929), all of whom showed that the isolated, perfused, hind limb of the dog would remove fructose from the perfusion fluid and therefore at least implied that this sugar was metabolized in the absence of the abdominal viscera. Griffiths and Waters (1936) had also found that fructose prolonged the life of the eviscerated dog, again indicating utilization, but these experiments may have been influenced by the presence of the kidneys which were not removed.

The destruction of fructose by the nephrectomized, eviscerated rat. In view of the contradiction implied in the reports mentioned and since the chief weakness of the work of Corkill and Nelson lay in their indirect estimation of the fructose content of their preparation, it seemed desirable to reinvestigate the problem from their viewpoint, but using the rat, which is small enough to be analyzed in toto.

Adult, male rats were eviscerated (the alimentary tract from esophagus to lower colon, pancreas, spleen and liver were removed) after a preliminary, partial ligation of the vena cava (Markowitz and Soskin, 1927) and then nephrectomized in such a manner as to leave the suprarenals. The animals were given 200 mgm. of fructose and the same amount of glucose via the saphenous vein immediately after the completion of surgery and at intervals as indicated for each animal in figure 1. The glucose was given to make certain that the animals would live long enough to allow their tissues ample time to act on the fructose if they were so

[1] Excerpt from thesis presented to Graduate School of the University of Minnesota in partial fulfillment of the requirements of the Ph.D. degree in physiology. Supported in part by a Sigma Xi Grant-in-Aid. Assistance in the preparation of these materials was furnished by the personnel of the Works Progress Administration, Official Project no. 665-71-3-69, Subproject no. 355.

able. After two or three injections the animals were allowed to go on to death without further sugar. Blood samples were taken from the tip of the tail throughout the period of survival, and each sample analyzed for both fructose (Reinecke) and total sugar. The total sugar analyses were made by a modified form of Jeghers and Myers' method (1930) which was found to give the same values for fructose and glucose. The difference between the fructose and total sugar values of a sample was taken as its glucose content.

Immediately after death each animal was dropped into enough hot water containing 1 equivalent of sulfuric acid to make a total volume, including that of the animal's carcass, of approximately 1 liter, and digested by boiling under reflux for 4 hours. These digests were analyzed for fructose as follows: a 10 ml. aliquot was measured into a 100 ml. graduated cylinder containing 20 ml. of 10 per cent

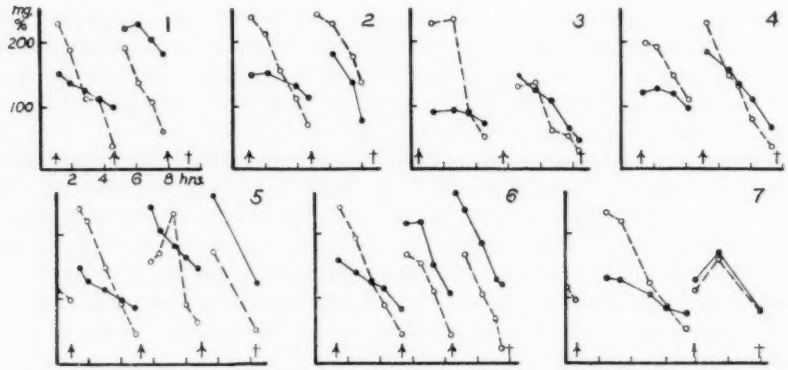

Fig. 1. The blood fructose and glucose levels of eviscerated, nephrectomized rats after the intravenous administration of these sugars. Solid lines indicate fructose levels, broken lines glucose levels. Each arrow indicates the injection of 200 mgm. of fructose and a like amount of glucose. The crosses indicate the death of the respective animals. The numbers in the upper right hand corners identify the animal concerned for comparison with figure 2, group A.

zinc sulfate and a drop of phenolphthalein. Water was added to make 50 ml. and then a saturated barium hydroxide solution to a faint but permanent pink endpoint. Finally more water was added to make 100 ml. and a portion centrifuged. The supernatant fluid was decanted and acidified with a trace of concentrated sulfuric acid. If barium sulfate was formed, it was removed by recentrifugation. Fructose was determined in the clear fluid by the method mentioned. Analyses of controls which had not received fructose showed only a small amount of material giving a reaction for fructose. Analyses of similar controls which were killed within a few minutes after receiving fructose showed that the method detected slightly more than 50 per cent of the fructose present. That the missing fructose was destroyed by the acid treatment is shown by the disappearance of slightly less than 50 per cent of known amounts of fructose boiled under reflux with normal sulfuric acid for the same length of time (see

fig. 2, groups B, C and D). It was felt that such drastic treatment was desirable in order to avoid the objection that fructose stored in some form capable of being rehydrolyzed to fructose might have been missed.

Figure 1 shows that concentration in the blood of both the fructose and glucose in these animals decreased with time, the rate of decrease of fructose sometimes approaching that of glucose. That the decrease in the blood fructos-levels reflected a real loss of fructose from the entire system of tissues was shown

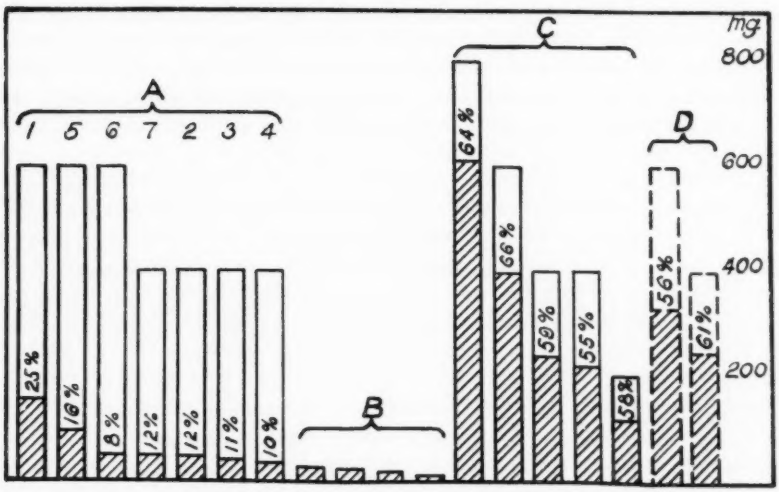

Fig. 2. Analyses of rat carcasses for fructose. The total height of each bar indicates the quantity of fructose added to the system, the shaded portion the amount accounted for by analysis. The percentage accounted for is given at the top of each shaded bar. Group A gives the analyses of the animals whose blood sugar curves are given in figure 1. The numbers above the bars identify them for comparison. Group B gives analyses of controls given no fructose, group C of controls killed a few minutes after the injection of known quantities of fructose. Group D shows the results of the analysis of solutions containing known quantities of fructose in normal sulfuric acid which had been boiled under reflux for the usual digestion period. These analyses were carried through the complete procedure, but did not differ significantly from analyses on aliquots in which the treatment with zinc sulfate and barium hydroxide was omitted.

by the results of the carcass analyses (fig. 2, group A) which accounted for much less than the 50 per cent of fructose injected as found in the controls. In fact the amount of fructose detected in some cases approached that found in the controls which had received no sugar. The highest proportion found, 25 per cent, was in an animal which died about an hour after an injection of the sugar.

The influence of fructose on the length of survival of the eviscerated rat. The disappearance of fructose from the tissues of the eviscerated, nephrectomized rat does not in itself mean that it is metabolized in such a way as to be effective in supporting the life of the various tissues of this preparation. Of the reports men-

tioned above indicating that tissue systems can act on fructose in the absence of the liver and intestine only that of Griffiths and Waters, showing that it prolongs the life of the eviscerated, non-nephrectomized dog, implies directly that it is used in the support of tissue metabolism. Therefore experiments were carried out in the eviscerated rat to determine whether or not fructose would also prolong the life of this preparation. These were carried out on groups of 4 adult, male, eviscerated rats, 2 nephrectomized and 2 with kidneys intact. The animals were fasted for approximately 24 hours before being eviscerated. They were given 2 ml. of saline intravenously immediately after the completion of the surgical procedures. One hour later, one nephrectomized and one non-nephrectomized animal were given 0.8 ml. of 50 per cent fructose intravenously followed by 0.25 ml. of saline. The other two were each given 1.05 ml. of saline. All of these fluids were given through a slender rubber tube, anchored at the back of the

TABLE 1

The survival time in minutes after the completion of surgery of eviscerated rats treated and not treated with fructose

GROUP	NEPHRECTOMIZED		NON-NEPHRECTOMIZED	
	Fructose not given	Fructose given	Fructose not given	Fructose given
I	95	107	232	444
II	120	82	303	883
III	93	159	397	574
IV			415	675
			485	
V*			795	995

* Blood samples were obtained at intervals from the tips of the tails of all animals in groups I–IV, care being taken to obtain the samples in the same way and at the same relative time for all animals within a group. No blood samples were taken from the animals in group V which may account for their markedly longer survival.

neck by a suture and leading under the skin to a cannula tied in a jugular vein. The amount of fluid injected was sufficient to cause the excretion of urine in the non-nephrectomized animals. The animals were then allowed to go on to death without the administration of further saline or sugar. The survival times of the animals are shown in table 1. It is felt that only animals within a group should be compared, for the experiments on different groups were carried out on different days, and it was found impossible to control all of the conditions of the experiments precisely from day to day.

The table shows that the non-nephrectomized animals receiving fructose consistently lived longer under the circumstances than did their controls. It also shows that other things being equal the non-nephrectomized lived longer than the nephrectomized, eviscerated rat. The survival times of the nephrectomized animals were too short and too irregular under the conditions of these experiments to allow the drawing of any conclusions as to the effect of fructose in these ani-

mals. Therefore they were not included when the experiments were performed on the later groups.

Discussion. The finding that fructose disappears from the eviscerated, nephrectomized rats seems to be contradictory to the findings of Corkill and Nelson; but it is to be pointed out that they were working with a different species, performed a somewhat different type of experiment and estimated the amount of fructose remaining in their preparation by an indirect means. The reports of the workers using the perfused hind limb of the dog are supported.

The prolongation of life of the eviscerated, non-nephrectomized rat by the injection of fructose supports the similar finding of Griffiths and Waters for the dog and indicates the probability that fructose is metabolized in such a way as to support the life of tissues, at least in the non-nephrectomized, eviscerated rat.

The rapid demise of the nephrectomized as compared with the non-nephrectomized, eviscerated rat may be explained by the finding of Bergman and Drury (1938) that it was necessary to inject glucose more rapidly into a nephrectomized or anuric, non-nephrectomized, eviscerated rabbit to maintain its blood sugar at a given level than into a similar animal with functioning kidneys. The excretion of urine by all the non-nephrectomized animals makes it unlikely that a difference in kidney function could explain the longer survival of the non-nephrectomized animals receiving fructose as compared with their controls.

The results of these experiments neither support nor contradict the finding of Bollman and Mann (1931) that fructose would not prevent or relieve the symptoms of hypoglycemia in the eviscerated, non-nephrectomized dog or that of Maddock, Hawkins and Holmes (1939) that it would not return to normal the electroencephalogram of the hypoglycemic, eviscerated, non-nephrectomized rabbit. In the studies of these investigators apparently no effort was made to detect any difference in length of survival of eviscerated animals injected with fructose as compared with controls receiving no sugar, or to account for all the fructose at the end of the experiment. On the other hand, in the experiments here reported no attempt was made to prevent the ultimate occurrence of hypoglycemic symptoms by continued injection of fructose into the non-nephrectomized preparation or to relieve them after they had begun. These symptoms, however, did not occur as soon in the animals receiving fructose as in those not receiving this sugar.

SUMMARY

Evidence has been presented which indicates that the eviscerated, nephrectomized rat can destroy fructose.

Fructose was found to prolong the life of the eviscerated, non-nephrectomized rat.

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THE EFFECT OF THE URETHANE OF BETA METHYLCHOLINE CHLORIDE UPON THE PARASYMPATHECTOMIZED CAT'S EYE

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Shen and Cannon (1) demonstrated that excision of the ciliary ganglion produces a pupil which is sensitized to instilled acetylcholine. This finding was confirmed by Keil and Root (2) who also found that a similar phenomenon follows intracranial section of the oculomotor nerve. According to the latter investigators the contraction of the iris sphincter in response to a given dose of acetylcholine reaches a maximum in about five days, and similar curves are obtained until at least the eighteenth day. After this time the responses decrease until they reach a steady minimum within approximately thirty-five days. The recovery of acetylcholine sensitization which occurs when the drug is injected following physostigmine instillation as well as certain observations in the literature (2) suggest that the loss of sensitization in parasympathetically denervated pupils is associated with an increased choline esterase activity.

It should be possible to test this hypothesis by injecting a drug which possesses the muscarine-like activity of acetylcholine, but which is not destroyed by choline esterase. Such a substance was sought among the choline derivatives, for these are known to vary in activity and stability (3, 4, 5). At the suggestion of Dr. Hans Molitor, the urethane of beta methylcholine chloride[2] (hereafter designated Ubm) has been used. This substance has ten times the muscarinic activity of acetylcholine, and it is said to be stable in the presence of choline esterase (6).

METHODS. All operations were carried out with strict aseptic precautions on cats anesthetized with nembutal (36 mgm. per kgm. body weight, intraperitoneally). Denervation of the sphincter was accomplished by excision of the ciliary ganglion (1).

At various intervals after operation the responses of the circular muscle of the iris to the intravenous injection of Ubm were observed. Changes in pupillary size were determined by measuring the horizontal diameter with a millimeter scale. From time to time the pupil on the side of the ciliary ganglionectomy was tested for regeneration by instilling into the conjunctival sac two drops of a one per cent solution of physostigmine. When physostigmine instillation produced constriction of the pupil seven days after removal of the ciliary ganglion, the

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[2] The authors wish to thank Dr. Hans Molitor and the Merck Institute for Therapeutic Research for supplying the urethane of beta methylcholine chloride used in these experiments.

excision was assumed to be incomplete. A positive physostigmine effect six weeks or more after ciliary ganglionectomy suggested regeneration of the parasympathetic innervation. Experiments in which there was any suspicion of incomplete removal of the ganglion, or regeneration of the parasympathetic nerve fibers were discarded.

RESULTS. The normally innervated cat's pupil constricted when 1 or more mgm. per kgm. body weight of Ubm were injected intravenously (3 cats). Two to six hours after the drug was injected the miosis disappeared. The injection of these large amounts of Ubm produced a pupillary constriction of the parasympathetically denervated pupil at a rate and to a degree that was nicely reproducible for a period of at least seven to eight weeks. In two cats the time required for the maximum contraction of the sphincter increased slightly in the later curves. This change was never great.

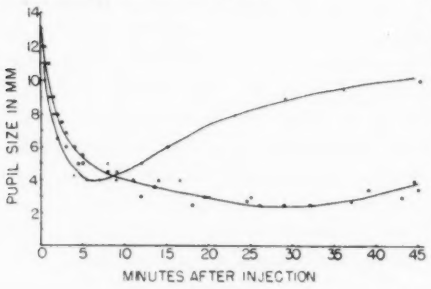

Fig. 1

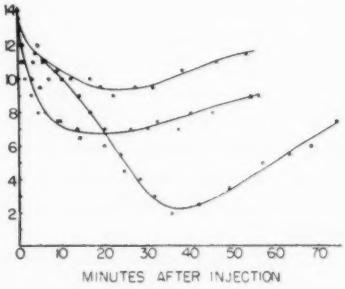

Fig. 2

Fig. 1. The response of the left pupil to the intravenous injection of the urethane of beta methylcholine chloride (115 gamma per kgm. body weight) 8 (+), 15 (△), 21 (●), and 28 (○) days after excision of the left ciliary ganglion.

Fig. 2. The responses of the left pupil to the intravenous injection of acetylcholine (5 mgm. per kgm. of body weight) and of the urethane of beta methylcholine chloride (150 gamma per kgm. of body weight). The acetylcholine was injected 8 (○) and 34 (●) days after excision of the ciliary ganglion; the urethane derivative, 12 (×) and 44 (△) days after the operation.

The injection of smaller quantities of Ubm (100 to 200 gamma per kgm. of body weight) produced a contraction of the denervated sphincter (9 cats), whereas little change occurred on the normal side (paradoxical pupillary constriction). Figure 1 shows the pupillary constriction which is induced by the intravenous injection of 115 gamma per kgm. of body weight of Ubm 8, 15, 21 and 28 days after excision of the ciliary ganglion. It is important to note that the curves obtained 15, 21 and 28 days after the operation show no diminution in the magnitude of the response. This was true also in cats in which after ciliary ganglionectomy the test injections were carried out for a period of six weeks. In the four animals in which the first test injections were made between 3 and 8 days after excision of the ciliary ganglion, the rate of pupillary constriction and the rate of recovery were more rapid than was the case in the later curves (see fig. 1). There is no obvious explanation for this phenomenon.

The minimum dose of Ubm necessary for the production of contraction of the denervated sphincter varied from animal to animal. Thus, the injection of 120 gamma per kgm. of body weight produced no pupillary constriction in one cat, but 240 gamma induced a good response. In a second animal 94 gamma of the drug were without effect, whereas 185 gamma resulted in a large constriction. In four other cats test doses of 100, 115, 136 and 137 gamma per kgm. of body weight were adequate amounts of Ubm for the production of reproducible pupillary constriction. These results indicate that in cats the threshold probably varies between 90 and 125 gamma of Ubm.

Since the above experiments show that the constriction of the parasympathetically denervated pupil which follows the intravenous injection of a given dose of Ubm can be reproduced for several weeks, and since the responses of a similar preparation to intravenous test injections of acetylcholine decrease about three weeks after ciliary ganglionectomy (2), it seemed of considerable interest to study the effect of the injection of these drugs upon the same denervated sphincter. Figure 2 shows that the pupillary constriction produced by the intravenous injection of 5 mgm. of acetylcholine per kgm. of body weight was much less 34 days after ciliary ganglionectomy than the response to the same dose which was obtained 8 days after the operation (2). On the other hand, the pupillary constriction which follows the intravenous injection of 150 gamma per kgm. of body weight of Ubm was as great 44 days after parasympathetic denervation of the eye as that obtained 12 days after ciliary ganglionectomy. This experiment demonstrates that the contraction of the denervated sphincter of the cat's eye which follows the intravenous injection of Ubm can be induced with undiminished intensity at a time when the response of the same pupil to the intravenous injection of acetylcholine has decreased.

Certain systemic reactions were observed immediately after the intravenous injection of Ubm. Clonic and tonic convulsions appeared only when the doses injected were greater than 1 mgm. per kgm. of body weight. In no instance was artificial respiration necessary. Salivation and lachrymation were more intense and more prolonged than when acetylcholine was injected. Dyspnea was present, but sweating of the foot pads was not observed. Urination and defecation occurred when larger doses of Ubm were injected.

Discussion. The constriction of the normally innervated pupil in response to the intravenous injection of large doses of Ubm indicates that this drug possesses a miotic action which is more intense than that of acetylcholine (2). Indeed, the miotic activity of Ubm appears to be comparable with that shown by natural muscarine (7). Since the normally innervated pupil constricts when large doses of Ubm are injected, denervation sensitization can be demonstrated only when small quantities of the drug are administered. Under these circumstances the denervated sphincter contracts, whereas little or no change occurs on the normal side (paradoxical pupillary constriction).

The miotic activity as well as the intense salivation and lachrymation which follow the injection of Ubm indicate that this drug has a more intense muscarine-

like action than does acetylcholine. In addition, Ubm is said to be stable in the presence of choline esterase (6). The difference in stability of the two drugs could explain the finding that Ubm produces a constriction of the parasympathetically denervated pupil which can be obtained with undiminished intensity at a time when the response of the same pupil to the intravenous injection of acetylcholine has decreased. This observation supports the conception that the decrease in acetylcholine sensitivity which occurs three weeks or more after ciliary ganglionectomy is related to an increased activity of the esterase system (2).

SUMMARY

1. The normally innervated cat's pupil constricts when one or more mgm. per kgm. body weight of the urethane of beta methylcholine chloride are injected intravenously (3 cats).

2. The intravenous injection of 100 to 200 gamma per kgm. of body weight of the urethane of beta methylcholine produces a constriction of the parasympathetically denervated pupil (9 cats), whereas little change occurs on the normal side (paradoxical pupillary constriction).

3. The intravenous injection of an adequate dose of the urethane of beta methylcholine chloride produces pupillary constriction curves which, between two and six weeks after ciliary ganglionectomy, do not vary significantly in the degree of contraction of the sphincter, or in the rate at which the contraction occurs.

4. The minimum effective dose of the urethane of beta methylcholine which produces constriction of the denervated sphincter probably varies between 90 to 125 gamma per kgm. of body weight.

5. The contraction of the denervated sphincter of the cat's eye in response to the intravenous injection of an adequate dose of the urethane of beta methylcholine can be produced with undiminished intensity at a time when the response of the same pupil to the intravenous injection of acetylcholine (5 mgm. per kgm. of body weight) has decreased (fig. 2).

6. These experiments support the conception that the decrease in acetylcholine sensitivity which occurs three or more weeks after ciliary ganglionectomy is related to an increased activity of the choline esterase system.

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THE CHANGES PRODUCED ON THE OXYGEN AND CARBON DIOXIDE CONTENT OF ARTERIAL AND VENOUS BLOOD OF THE BRAIN DURING DIATHERMY THERAPY FOR GENERAL PARESIS

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On the basis of his results with artificially induced fever in dogs Hartman (1, 2) has concluded that a constant and severe anoxia occurs which results in brain lesions similar to those of a prolonged asphyxia or carbon monoxide poisoning. He found that in all but one of his experimental animals the oxygen saturation of the arterial blood fell below normal and that the five animals in which the saturation fell below 65 volumes per cent all died. To overcome this anoxia he suggests that oxygen should be administered during fever therapy.

We have studied the changes produced in the blood constituents from 12 patients during diathermic fever treatments for general paresis. The determinations were made on blood taken from the radial artery and the internal jugular vein before therapy started, at the end of two and a half hours when the temperature reached 106°F., and after holding the temperature at this level for 3 to 4 hours. The following factors were studied: oxygen, carbon dioxide, sugar, lactic acid, total protein, specific gravity, hematocrit values, and pH. The pulse, respiration and blood pressure were measured and the rectal temperature recorded continuously on a Leeds and Northrup recording thermometer. In all but three instances the determinations were repeated. Two of these patients died prior to a second experiment. The results obtained on these two patients will be discussed later.

Readings were obtained in duplicate on oxygen and carbon dioxide of both venous and arterial blood for twenty-one experiments and in twenty of these the oxygen capacity was also determined. The means and standard deviations for the three readings of the variables studied are given in table 1.

It will be noted that from the first to the second reading there was a slight fall in the oxygen content of both the arterial and venous blood samples, but that the third reading showed an increase to an even higher level than at the initial reading. The oxygen saturation showed a similar fall and rise but the final value remained lower than the initial level. Part of the increase in oxygen content for the third reading was due no doubt to the increased hemo-concentration as the hematocrit values showed an increase from 40.01 per cent to 43.04 per cent which indicated a 7.5 per cent loss of fluid from the vascular system. As the

temperature was raised there was also an increase in the variability of the readings for both the absolute level of oxygen and the per cent of saturation in the arterial and venous blood samples. This change in variability was most marked in the values for percentage saturation of the arterial bloods taken at the first and second readings, the values for the standard deviations during this time increasing from 6.96 per cent to 14.59 per cent.

The carbon dioxide content of both the arterial and venous blood samples showed a progressive fall amounting to 4.16 volumes per cent for the former and 4.31 volumes per cent for the latter.

The initial temperatures of the patients were quite uniform as indicated by the low values for the standard deviations. That the temperatures were held within

TABLE 1

Means, standard deviations and ranges for all variables for three periods

		FIRST READING			SECOND READING			THIRD READING		
		Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range
Temperature, °F.		98.41	0.700*	97.4–100.8	105.95	0.389	105.0–106.5	105.92	0.358	105.2–106.6
Pulse		73.9	14.00	56–112	112.1	18.21	76–150	116.3	15.32	88–148
Respiration		18.8	3.68	14–28	23.9	5.63	16–38	23.4	4.42	16–34
Blood pressure, mm. Hg	Syst.	118.5	17.21	86–156	126.5	13.15	106–144	110.4	13.65	88–130
	Diast.	64.9	18.11	54–100	36.0	29.62	0–78	38.9	32.47	0–96
Carbon dioxide, vol. per cent	Art.	51.27	3.29	44.58–57.50	49.86	4.05	42.61–59.63	47.11	3.14	39.64–51.49
	Ven.	57.21	3.51	49.66–62.92	56.28	5.26	44.78–67.78	52.90	4.68	38.29–59.02
Oxygen, vol. per cent	Art.	16.28	2.08	12.41–19.59	15.81	3.03	10.25–20.88	17.01	3.03	10.69–21.89
	Ven.	10.25	2.64	5.34–17.24	9.85	3.08	6.02–18.09	11.47	3.51	7.94–22.48
Oxygen capacity, vol. per cent		18.16	2.54	14.63–23.35	18.90	2.19	14.42–22.77	19.84	2.69	14.50–24.70
Oxygen, per cent saturation	Art.	90.20	6.96	72.7–100.0	83.60	14.59	45.1–100.0	85.90	11.29	53.6–100.0
	Ven.	58.10	13.38	32.0–97.3	51.10	14.60	20.2–88.5	57.70	14.17	37.5–92.7
Hematocrit per cent	Ven.	40.01	3.53	32.2–44.6	41.42	4.19	33.8–47.2	43.04	5.42	32.0–49.9
A-V difference, carbon dioxide, vol. per cent		−5.95	1.94	−9.95–−0.78	−6.42	3.54	−12.24–+0.68	−5.79	3.23	−10.23–0
A-V difference, oxygen, vol. per cent		6.03	2.59	10.39–0	5.97	3.22	11.57–0.32	5.55	3.35	11.59–0

* The standard errors may be calculated from these values by dividing by $\sqrt{n-1}$ or in these experiments by 4.47.

very narrow limits during the period of elevation is shown by the almost identical means and standard deviations for the last two periods. In only two of the 38 readings were values below 105.5°F. obtained, one a reading of 105.0° and the other 105.2°. The upper limit of temperature exceeds 106.3°in only three instances. During the period of elevated temperature the variability was about one-half that found for the initial reading.

As the temperature was elevated the mean pulse rate increased from 73.9 to 112.1, and then to 116.3. The rate showed a significant positive correlation with temperature with a "r" of 0.709 and a probability of such a relationship occurring by chance of less than 1 in 100. This was a change of 5.1 beats per degree F. as compared with 7 beats found by Simpson (3). Even holding the temperature at this level for 3 to 4 hours caused no significant difference in the result as the value for the rate of change for the final reading was 5.6 beats per degree F.

Coincident with the change in pulse rate the mean systolic blood pressure also showed an increase of 8 mm. Hg for the period during which the temperature was increasing but this was followed by a marked fall during the period of elevation so that the final reading was 8 mm. Hg lower than the initial value. While the increase in temperature caused an increase in systolic blood pressure it had the opposite effect on the diastolic pressure which showed a drop from 64.9 mm. to 36.0 mm. from the first to the second readings and a slight rise to 38.9 for the final reading. The high standard deviations in the periods of elevated temperature were due to the fact that six patients gave at least one reading in which the diastolic pressure was listed as zero. These low pressures were undoubtedly caused by the extreme vasodilatation of the peripheral vessels which permitted a rapid fall in the blood pressure during diastole since the resistance to the flow of blood became negligible. As a result of these diverse effects on systolic and diastolic pressures the pulse pressures showed a very large increase from 53.6 mm. Hg before treatment started, to a maximum of 90.5 mm. Hg during the second reading. For the final reading some slight deleterious effect of the prolonged elevation of temperature is shown in the drop in the systolic pressure to a value somewhat lower than that of the control value. This produced a change in the pulse pressure to 71.5 mm. Hg which was still considerably higher than the initial value. The mean respiration rate also increased during the period of fever from 18.8 at the first reading to 23.9 for the second reading and 23.4 for the final reading. The coefficient of correlation between temperature and respiration was 0.52 and the Fisher's p for this occurring by chance was less than 0.01. The blood velocity was not measured directly but it is evident from the increases in pulse rate and pulse pressure that the velocity was markedly accelerated during the period of fever.

The differences between the arterial and venous levels for carbon dioxide and oxygen are also given in table 1.

Discussion. Our results on the effect of elevated temperatures on oxygen utilization do not agree with those reported by Himwich and his co-workers (4). They found that in 11 of 15 experiments the oxygen A-V difference rose from 2.0 volumes per cent to 8.1 volumes per cent. We found that the mean oxygen A-V difference changed from 6.03 volumes per cent for the control period to 5.97 volumes per cent for the second period and 5.55 volumes per cent for the final period. Of the 21 cases the second reading was higher than the first in 10 instances. There was no significant difference between any of the means, but there was a tendency to a slight but progressive decrease in the utilization of oxygen as the temperature was kept elevated. There was no significance in the differences between the arterial and venous values of carbon dioxide for the three readings but it is to be noted that the changes in carbon dioxide did not coincide with those of oxygen as the greatest difference was obtained between the first and the second periods. It is evident therefore that this change in carbon dioxide did not arise from an increase in metabolism That the increased rate of respiration has resulted in a washing out of carbon dioxide is shown by the progressive fall in both arterial and venous carbon dioxide content amounting to 4.16 volumes per cent for the former and 4.31 volumes per cent for the latter. The R.Q. as cal-

culated from the blood gas differences changed from 0.988 for the initial reading to 1.075 for the second reading, and 1.043 for the final reading. This would seem to indicate that the formation of fatty substances was accelerated in the brain during the period of elevated temperature.

Two of the patients studied in this series died. One of these deaths may possibly have been due to the diathermy treatment. He was given a single treatment during which his temperature rose from 98.2 to 106.2 within an hour and remained elevated for 4 hours. His pulse increased from 56 to 76 and then to 98 beats per minute and his blood pressure first rose from 138/68 to 145/50 and then fell to 100/0. The respiration rate increased from 18 to 24 and then to 28 per minute. The arterial blood oxygen levels were 18.02, 18.41 and 18.95 volumes per cent for the three samples and the corresponding percentages of saturation were 93.3, 90.1 and 92.7. The venous blood-oxygen levels were 10.78, 18.09 and 18.95. The pH values were 7.32 for venous and 7.37 for arterial at the beginning, 7.44 for both at the second period, and 7.42 for both at the third period. It is evident therefore that despite the fact that his blood was being oxygenated sufficiently in its passage through the lungs, it was not delivering any oxygen to the brain cells. This is also shown by the fact that the pH of the venous and arterial bloods were identical for the last two periods. The change in carbon dioxide content of the blood also substantiates this. The initial reading gave 57.04 volumes per cent for venous blood, and 47.51 volumes per cent for arterial blood. The next two readings show no significant difference between the arterial and of the venous bloods, the values for the second reading being venous 46.88 volumes per cent and arterial 46.38 volumes per cent, and those for the final reading being 45.92 and 45.82 volumes per cent. The day following this treatment the patient had an N.P.N. of 60.0 mgm. per cent, 6 days later the level was 98.4 mgm. per cent and 8 days later just before his death the value was 142.0 mgm. per cent. The autopsy confirmed the finding of death from uremia. Unfortunately permission to examine the brain could not be obtained.

In the second case the death occurred from bronchopneumonia 52 days after the diathermy treatment which was the second one that the patient had taken. This patient showed a progressive decrease in the percentage saturation of the arterial blood from 94.2 per cent to 89.0 per cent and finally to 66.7 per cent. He also showed a decrease in the utilization of oxygen by the brain from 10.39 volumes per cent for the first period to 6.52 volumes per cent for the second period and no utilization during the third period. In this last period the pH of both venous and arterial blood had the same value, 7.54. It is questionable that this death can in any way be attributed to the diathermy treatment, but it is interesting to note that the two patients who died were the only ones who gave identical values for the pH of venous and arterial blood.

We have not been able to confirm the findings of Hartman (2) that oxygen saturation of arterial blood below 60 per cent results in death. In four instances we found arterial oxygen saturation values below 60 per cent. None of these patients showed any ill effect from the treatment and the patient who died as

the result of treatment had an oxygen saturation of over 90 per cent in his arterial blood.

In only 6 of 42 readings taken during fever was the arterial saturation below 70 per cent and the mean value for this period was approximately 85 per cent. Furthermore the decrease in the amount of oxygen supplied to the brain was not significant. This finding casts doubt upon the advisability of following Hartman's (2) suggestion that oxygen should be administered during fever therapy. The possibility that a partial anoxia occurs in the brain and other tissues during fever therapy and that in some cases this may be severe enough to result in death is not ruled out by these experiments but this cannot be controlled by inhalations of oxygen. The patient whose death may be attributed to the treatment certainly showed no utilization of oxygen by the brain during the treatment. However, more damage seemed to be caused to the kidneys than to the brain. A possible explanation for the effect on the kidneys may lie in the placement of the electrodes around the trunk so that a concentration of the heating effect occurred in the region of the kidney.

SUMMARY

In twelve patients receiving diathermy treatments for general paresis the oxygen and carbon dioxide levels of arterial blood and of blood from the internal jugular veins were determined. The temperature was raised to 106.0°F. and maintained at this level for 3 to 4 hours. No significant change in the mean A-V values for either carbon dioxide or oxygen was found. The mean oxygen saturation of the arterial blood decreased from 90.2 per cent to a minimum of 83.6 per cent. There was no evidence of increase in brain metabolism shown by the changes in the blood gases. A significant fall in the mean carbon dioxide levels of both arterial and venous blood occurred.

The administration of oxygen to patients during diathermy treatments does not seem to be warranted as the oxygenation of the arterial blood in the lungs is not dangerously lowered.

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AN ANALYSIS OF THE ACTION OF ACETYLCHOLINE ON THE CARDIAC GANGLION OF LIMULUS POLYPHEMUS[1]

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The following report deals with the response of the heart of Limulus polyphemus to certain choline derivatives, notably acetylcholine; it adds to the rapidly accumulating data which must be correlated in the ultimate evaluation of the rôle which this substance plays in the process of stimulation and transmission—especially its rôle in ganglionic activity.

This study has been made by the general methods used by Carlson (3) in his study of the action of drugs on this organ. This heart is neurogenic and the ganglion, owing to its superficial location in the dorsal median line of the posterior muscular segments, may be treated with solutions either *in situ* or after it has been dissected free from underlying tissue. The anterior (first and second) segments possess no rhythmogenic nerve cells, normally exhibit no inherent rhythm and contract solely in response to impulses from the ganglion. These anterior segments, therefore, like a nerve-muscle preparation, may be directly studied for effects upon the neuro-muscular transmission and graphic records of their contraction serve to quantitate the muscular responses to the efferent impulses from the ganglion.

Studies were made with acetylcholine, either as bromide or chloride, with acetyl-beta-methylcholine chloride ("mecholyl") and with carbaminoylcholine chloride ("lentin," "doryl").[2] Their effects were compared with the action of choline bromide. The drugs were dissolved in blood plasma or in sea water—in a few instances in Van't Hoff's solution. It was found that solution of the drugs caused only slight changes in pH which were wholly without physiological significance. All solutions were freshly prepared for each experiment and careful checks were made to give assurance that the hydrolytic decomposition during the time course of any experiment was quantitatively negligible. The responses of eighty-nine hearts were observed.

NEURO-MYAL TRANSMISSION. The anterior segments were treated with the desired solution by immersion, by lavage, or as in some of our experiments by perfusion through the lumen. Special precautions were taken to avoid all

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[2] The author is indebted to Merck and Company for an adequate supply of crystalline "lentin".

possible contamination of the posterior ganglionated portion of the heart and thus to limit the action of the solutions strictly to the neuro-myal structures. This can be done either with the heart *in situ* in the animal or with that organ removed from the body. All methods gave identical experimental results which may be briefly summed in the statement that neither acetylcholine nor the other choline derivatives mentioned above when applied to the muscle alone had the slightest stimulating effect either upon the rate or the height of contraction of the normally beating heart (fig. 1), even when the concentrations in the sea water or Van't Hoff's solution were excessively high (1 in 100). The slight weakening of the muscular contraction, sometimes noted, is quite in contrast with the stimulating effect of the solution diluted forty times (1 in 4000) when applied to the isolated ganglion of the same heart, for, as is usually the case with this

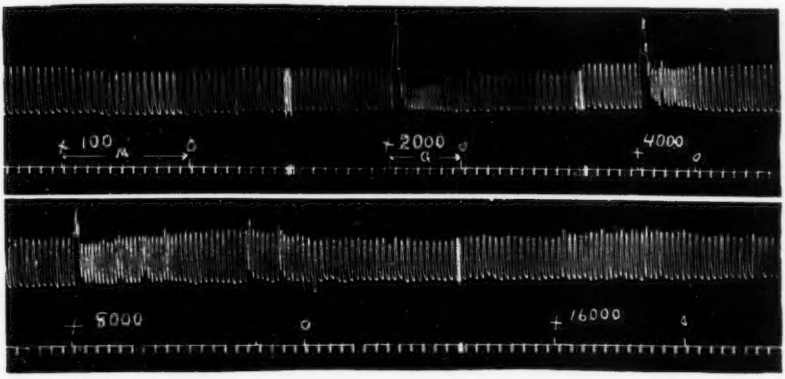

Fig. 1. Effects of acetylcholine on the heart of Limulus. The numerals indicate the dilution of 1 part of acetylcholine in sea water. + indicates the application and ○ the removal by washing with sea water. One part in 100 was applied to the muscle—M—of the anterior segments without physiological results. The more dilute solutions, 1 part in 2,000, 4,000, 8,000 and 16,000, were applied successively to the ganglion—G—and produced stimulation. No potentiation. Time trace, lower line—6 sec.

concentration, there was a sharp tetanic contraction of the muscle and accelerated rhythm; the decreased height of contractions often noted in the latter instance are secondary to the faster rate and are not due to ganglionic depression.

The above effects of acetylcholine were obtained in our earlier experiments without attempts at potentiation with eserine or prostigmine which is so essential to the stimulating effects of acetylcholine in the presence of cholinesterase. Contrary to the statements of Bacq (2), this specific enzyme has been demonstrated in Arthropods in the blood, skeletal muscle and heart muscle of Limulus by Smith and Glick (12) who have found the cardiac ganglion of Limulus to be especially rich in cholinesterase.

The experiments were therefore repeated in an effort to potentiate the acetylcholine effects with eserine and with prostigmine. These drugs were applied to the muscle of the anterior segments in concentrations far greater than was

necessary to inactivate all cholinesterase (1 in 1000 eserine) and subsequently the muscle was treated with acetylcholine bromide solutions in concentrations varying from one in one hundred thousand to one in one thousand without eliciting any alteration in the height of the normal muscular contractions. Similarly when acetylcholine and the potentiating drug were present in the same solution (sea water) there was no potentiation of contractions, nor did they elicit the characteristic contracture exhibited by the dorsal muscles of the leech and the rectus abdominis muscles of the frog.

The above series of experiments was repeated upon the severed anterior segments of the heart. Contractions were elicited by electrical stimulation of the dorsal median nerve fibers, a preparation in every way comparable to an ordinary nerve-muscle preparation. The muscle was immersed in solutions of the drugs, either separately or simultaneously, and induction shocks of various strengths and sequential groupings were applied to the dorsal median nerve of the preparation. Without further detail it may be stated that in no instance was there the slightest evidence of potentiation, or in fact of any action of acetylcholine on the contractions. Mecholyl and lentin were likewise without augmenting effect upon the muscular contractions which were induced by electrical stimulation of the nerve. Corroborative evidence is to be found in Carlson's (3) statement that physostigmine in high concentration (1 in 500) was without physiological effect on its contractions when applied to the heart muscles and further confirmation is found in the fact that mecholyl and lentin (doryl) likewise fail to enhance contractions of these muscles although they are practically unaffected by the hydrolytic action of cholinesterase. From these results it is obvious that the presence of cholinesterase is not *prima facie* evidence that the acetylcholine mechanism is present. This enzyme plays no rôle in the neural stimulation of the cardiac muscle of Limulus. Likewise, on the basis of the above results, acetylcholine can be eliminated from consideration as a chemical mediator of the nerve impulses to the heart muscle of Limulus.

As a corollary to these conclusions we may add that the above experiments indicate conclusively that there are no functionally rhythmogenic nerve cells in the first two segments, a fact long ago demonstrated by Carlson (1900) and abundantly supported by many observations since that time. The following sections will show that, were such nerve cells present, they would have been stimulated and aroused to rhythmic activity by the action of the choline esters used in the experiments recounted above.

Effects upon the ganglion. The ganglion was subjected to the action of the solutions of choline esters by lavage, by immersion of the posterior segments with its ganglion *in situ*, or by similar treatment of the ganglion after dissecting it free of the underlying muscle but retaining connection with the untreated anterior muscular segments, the contractions of which served as an index of ganglionic activity. Further details of method will be omitted since all methods gave identical effects both qualitatively and quantitatively. In effective concentrations, all the choline derivatives caused initial simulation of the ganglion, an effect which was sustained in solutions of moderate strength but with stronger

solutions there was frequently a subsequent decrease of rate and weakening of contractions which may be attributed to inhibitory action of the drug from which, however, there was ultimate recovery in sea water if the effect was not too profound. Inhibition as an initial effect of these drugs was never observed. In these features the action of the cholinesters was similar to that observed by Carlson (3) with other drugs which are known to inhibit the vertebrate heart such, e.g., as muscarine, pilocarpine, physostigmine and nicotine. In the experiments to be described we are not concerned with peripheral innervation but solely with effects upon the ganglion. By analogy with effects on the vertebrate sympathetic ganglia the effect of dilute solutions would be the "nicotine effect."

When acetylcholine was used without any previous treatment which might induce sensitization or potentiation, our experiments showed that 69 out of 73 tested for the liminal stimulation value of acetylcholine showed effects only when the concentrations were greater than one part in 16,000. In general the concentrations required were one in 10,000 of sea water, although eight of these hearts showed no response until the strength was one in 5,000 or greater. Of four very sensitive hearts, one showed a response of a limiting dilution of approximately 120,000, one at 50,000, one at 36,000 and one at 25,000. These concentrations are excessively high in comparison with those which are necessary to produce other known physiological effects of acetylcholine; for example, inhibition of the vertebrate heart (1 in many millions) and in almost unbelievable dilution ($1:10^9$ even $1:10^{12}$) it will inhibit the heart of the marine clam, Venus mercenaria (11). The conclusion that the ganglion of Limulus lacks sensitiveness to drugs, based on the above results, is negatived by Carlson's (3) evidence that while its stimulation requires relatively high concentrations of some drugs the ganglion can be stimulated by high dilutions of nicotine (1 in 5 million), aconitine, veratrine and adrenalin.

Figure 1 shows a series of tracings illustrating the effects of acetylcholine described above. In it one may note that even 1 to 100 acetylcholine failed to stimulate the muscle or the myoneural junctions of the second segment of the heart, while decreasing concentrations $\frac{1}{2000}$, $\frac{1}{4000}$ and $\frac{1}{8000}$, when applied to the ganglion initiated a transient tetanus followed by an accelerated rate with its consequent reduction in height of contraction. A dilution of $\frac{1}{16,000}$ caused mild stimulation noted in an increase of both rate and height of contraction. The stimulating effects of a $\frac{1}{24,000}$ dilution were just detectable. This liminal concentration must be looked upon as physiologically excessive but it may safely be said the ganglion of this particular heart would not have responded to it had it not experienced previous treatment with the stronger solutions of the drug. For most hearts the threshold for stimulating effects of lentin was reached at dilution approximating 1 part in 300,000.

Lentin (carbaminoylcholine chloride, "doryl"). Our own experiments show that the ganglion is especially sensitive to lentin (doryl), a compound closely related to acetylcholine, but one not hydrolyzed by cholinesterase. In general it may be said that lentin has more than ten times the stimulating activity of unpotentiated acetylcholine; frequently the individual ratio was as high as 25 to

1; to give a specific example, the most sensitive ganglion of our series responded to one part of acetylcholine in 120 thousand of sea water but a similar stimulation was induced by lentin, 1 part in 3 million. Lentin needs no potentiation, in fact cannot be potentiated by eserine, but lentin by itself has stimulating powers which closely approximate those of acetylcholine which has been potentiated with eserine (see below). There are certain differences, however. Strong solutions do not produce so stormy and powerful a tetanus, contractions are more regular, and augmentation of height usually accompanies the increase in rate, there being less tendency to the tonic contraction which raises the relaxation base.

Mecholyl chloride is a choline ester very slightly hydrolyzed by cholinesterase. It cannot be potentiated by eserine. Unlike lentin it has very slight stimulating value for the ganglion, possibly half that of unpotentiated acetylcholine and lacks entirely the "nicotine effect" of the latter drug. The base choline (bromide) also will stimulate the ganglion but is still less effective than mecholyl. Owing to the great variability in the stimulation threshold of different heart preparations it is impossible to fix definite values for the relative stimulating effects of these different preparations.

Potentiation to acetylcholine action. Reference has already been made to the fact that the cardiac ganglion of Limulus has a very high content of colinesterase (Smith and Glick, 12). It is well established that this enzyme, by hydrolytic decomposition of acetylcholine, can reduce materially or even abolish the physiological effects of this drug. Most effective in "paralyzing" this enzymic action are eserine (physostigmine) and prostigmine which thus "potentiate" the action of acetylcholine. This potentiating effect of eserine is especially well exemplified by its action on the Limulus heart ganglion. Although eserine itself does not stimulate the ganglionic discharge of the ganglion until it is present in concentrations greater than one part in 8,000 of sea water, solutions for more dilute than this, e.g., one in 50,000 or even one part in 100,000 of sea water (one part in 20,000 was commonly used in our experiments) leave the ganglion highly sensitive to subsequent dilutions of acetylcholine which without this potentiation have no detectable effect. The potentiation by eserine sets in almost instantaneously, and the effect of a single treatment for a few seconds may still be demonstrated several hours later in spite of persistent effort to wash the structure free from every trace of the potentiating drug. Prostigmine acts in a similar manner although there are quantitative differences which will not be dwelt with at this time. The effects of potentiation with eserine are illustrated in figure 2. In this experiment acetylcholine (1 in 10000) without potentiation produced no acceleration and 1 in 7500 merely effected a slight elevation of the basal relaxation tonus. Eserine alone in a concentration of 1 in 10,000 caused only mild stimulation (a 10 per cent increase in rate), but this single treatment profoundly affected the response to subsequent treatment with acetylcholine as is illustrated by the marked stimulation induced by this drug at a dilution of 1 in 50,000, a stimulation which is still exhibited with progressive dilution to 1 in 300,000.

Evidence of the marked potentiating action of eserine and the fact that this effect is wholly independent of its own stimulating value is presented in the trac-

ings in figure 3. In this illustrative experiment, on a highly sensitive preparation, both the eserine and acetylcholine were present in the stimulating solutions in equal concentrations, that of each being far below its individual stimulating value for the ganglion. While many other experiments demonstrated stimulation by such solutions when the concentrations were 1 in 100,000, in the example given above dilutions of 1 in 200,000 and 1 in 400,000 also produced marked stimulating effects.

Eserine and pilocarpine. The examples of stimulation shown in figure 3 serve another purpose; they indicate that eserine acts in a very specific way which is not the result of its own stimulating power. Its effectiveness is demonstrable

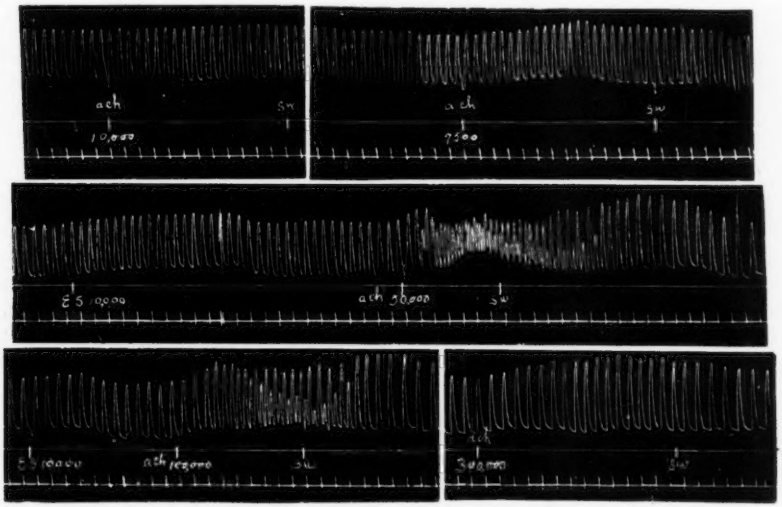

Fig. 2. Effects of potentiation of acetylcholine solutions with eserine upon their stimulation of the cardiac ganglion. Acetylcholine solutions 1 in 10,000 and 1 in 7,500 (upper tracing) produced only mild stimulation. Eserine (1 in 10,000), middle tracing, was only mildly stimulating but after its application, the stimulation by acetylcholine was demonstrable in high dilutions: 1 part in 50,000 (middle tracing) and 1 part in 100,000 and 300,000 respectively (lower tracing).

only in conjunction with acetylcholine. It does not have the slightest potentiating effects even in conjunction with choline or other choline esters such as mecholyl or lentin; this is an indication not only of its ineffectiveness as a direct stimulating agent at these dilutions but that its effect, furthermore, is not due to a rise in the threshold of stimulation, i.e., to an increase in the sensitiveness of the preparation to other stimulating agents. This pronouncement is best illustrated by the experiment reproduced in figure 3. The individual liminal stimulating concentrations for pilocarpine, eserine and acetylcholine differ very slightly for a given ganglion and in this preparation was a dilution of about 1 in 10,000 as shown in A and B of the figure. In C it may be seen that pilocarpine of this

liminal concentration does not potentiate a 1 in 10,000 solution of acetylcholine; neither does the combination of eserine and pilocarpine at D give any indication of enhancement of the stimulating power of either drug, but when eserine and acetylcholine (1 in 10,000) act together (E) a powerful irregular tetanus results, followed by an augmented rhythm, then a slow rate and final return to high regular contractions (not shown). This specific effect of the combination of these two drugs was evident with dilutions much greater than 1 in 400,000 (G); the same effect as that illustrated in figure 2.

ATROPINE. The action of this alkaloid was studied on account of its well known effect in "blocking" the action of acetylcholine in certain of its peripheral

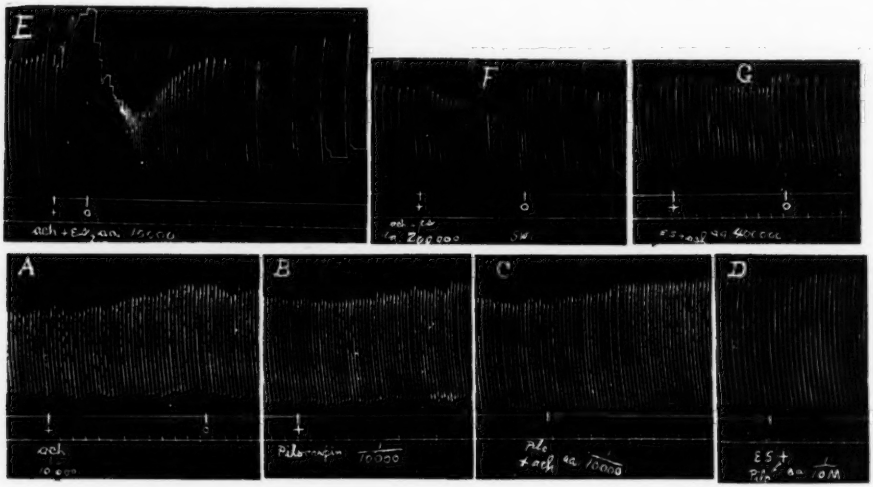

Fig. 3. In dilution of 1 part in 10,000 acetylcholine applied to the heart ganglion was only mildly stimulating (A) as was the same dilution of pilocarpine (B). The combination of these two agents at this dilution added little to the individual stimulation of either drug (C) and at (D) it is demonstrated that eserine, 1 part in 10,000, does not enhance the action of pilocarpine. The upper tracings show the marked stimulating action of equal parts of eserine and acetylcholine in dilutions of 1 in 10,000 (E), one in 200,000 (F) and 1 in 400,000 (G). + and ○ mark application and removal of the drugs, respectively.

effects such as vagal inhibition of cardiac muscle. Upon the cardiac ganglion of Limulus it has no such effect, its only action in non-toxic concentrations being stimulation, as Carlson (3) pointed out. The liminal concentration for this effect lies between 1 in 8,000 and 1 in 5,000. This stimulating effect increased with increasing concentration and could be summed with that of other stimulating drugs such as eserine and pilocarpine, but notably with unpotentiated acetylcholine itself, the effect of which upon the ganglion was never inhibited by atropine, thus comporting itself as it does on the sympathetic ganglia of vertebrates.

These facts emphasize the difference between the mechanism of inhibition of

the vertebrate heart and that of Limulus. In the latter, inhibition is restricted to the ganglion, which however is stimulated by acetylcholine, the peripheral motor neuromuscular function being unaffected by acetylcholine, while in the vertebrate heart it is the musculature which is inhibited by the action of the drug on parasympathetic nerve endings. In the ganglion there is no decrease in the stimulating power of potentiated acetylcholine. Figure 4 illustrates these points. A shows the stimulating effect of 1 in 2,000 atropine and the superimposed effect of 1 in 2,000 unpotentiated acetylcholine. In B, eserine and acetylcholine had been diluted to 1 in 100,000 and the atropine concentration increased in the common solution to 1 in 500, but the high stimulating potency of the dilute potentiated acetylcholine was not interfered with.

Inhibition. In this connection another point bearing on inhibition should be referred to, namely, that the stimulation of the ganglion by whatever agency used, particularly by potentiated acetylcholine (or by lentin), did not reduce the ease with which ganglionic inhibition could be induced by direct rapid faradiza-

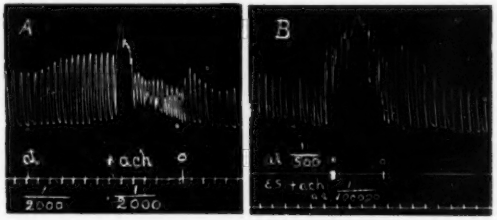

Fig. 4. Atropine is a stimulating agent when applied to the ganglion; a 1 in 2000 solution applied at *at* (A) increased the rate and amplitude of contractions, and stimulation by 1 in 2000 acetylcholine summed with it (+ to ○). Acetylcholine with potentiating eserine, each 1 in 100,000, produce profound stimulation of the ganglion in spite of atropine (1 in 500) B.

tion as described by Garrey and Knowlton (7). In fact the heightened activity of the ganglion actually made it easier to induce inhibition by direct electrical stimulation. This might have been anticipated on the basis of the Garrey and Knowlton observations. It is as if the stimulation by the drugs induced a condition tending to autogenous inhibiton and it is possible to so interpret either the weak contractions or the slow rhythm which so commonly accompanies or follows intensive stimulation by acetylcholine (fig. 3). Finally in this connection it should be stated that atropine does not interfere with inhibition of the ganglion however induced, whether by stimulation of ganglionic afferents, as has been done by Carlson, or by rapid repeated induction shocks or condenser discharges (7).

Resuscitation of ganglia. Hearts which have been left standing in sea water, or *in situ* in exsanguinated animals, often show marked irregularity of rate, with depressed contractions which have lost all semblance of uniformity. It was found in these cases that treatment with solutions of acetylcholine usually restored them to a marked degree. Two examples of this effect are shown in

figure 5, the contractions in A are those elicited by the depressed, exhausted or fatigued ganglion, they showed the immediate pick up which resulted when 1 in 10,000 acetylcholine was applied. Within ten minutes they were restored to regular uniform contractions and (B) the ganglion showed acceleration when treated with 1 in 200,000 acetylcholine after eserine potentiation, indicating a high degree of sensitivity and normal function. A less regular but nonetheless striking improvement in contraction of another exhausted preparation is illustrated in C (fig. 5) due to acetylcholine and also as a result of choline. The improvement remained effective only while these drugs were acting and dis-

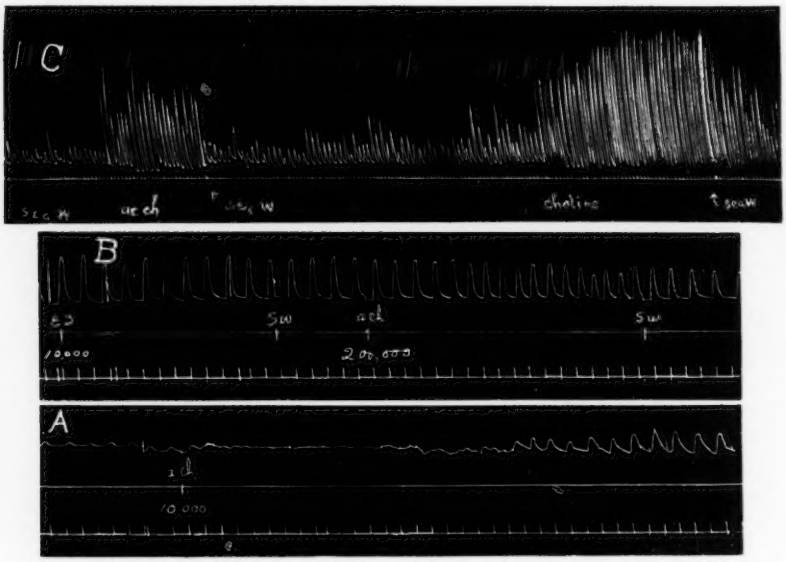

Fig. 5. Showing the muscular contractions initiated by an "exhausted" ganglion, A, and the prompt improvement due to 1 in 10,000 acetylcholine, illustrated by B; treatment with 1 in 10,000 eserine at *es* potentiated the ganglion to subsequent treatment with 1 in 200,000 acetylcholine solution which caused definite acceleration. The upper tracing (C) shows the improvement of contraction in another badly functioning ganglion by treating it with acetylcholine solution at *ach* and later also with a choline solution. Time tracing in 6 second intervals.

appeared when they were washed away. Had the treatment been continued it seems probable that restoration with complete regularity would have resulted. If vertebrate nerve centers are responsive to similar treatment these reactions assume a considerable significance. An earlier communication by Garrey (5) has pointed out that the only mechanism by which an increase in the height of contractions of the Limulus heart muscle can be accounted for is recruitment, i.e., the increase in the number of motor neurons delivering effective impulses to the muscle. The resuscitating effects then can only mean that within the ganglion more motor cells respond to the rhythmic impulses from the pace maker or, in

other words, either the receptive motor neurons or their synapses became more excitable as a result of the action of choline and its esters. This response will be referred to in a succeeding section. The results bear a striking resemblance to the neuromuscular augmentation, described by Garrey and Knowlton (6), which results from subliminal or very mild stimulation of the intracardiac nerve fibers and suggest that the augmentation described above is due to a stimulating action of acetylcholine on the motor cells of the ganglion and possibly is related indirectly to the "spontaneous background" discharge described by Armstrong, Maxfield, Prosser and Schoepfle (1).

SEGREGATION OF THE EFFECTS OF ACETYLCHOLINE STIMULATION. The outstanding expressions of stimulation are the increase in rate and the increase in the height of the contractions. While application of acetylcholine to the entire ganglion usually results in an increase in both rate and height it is usually possible

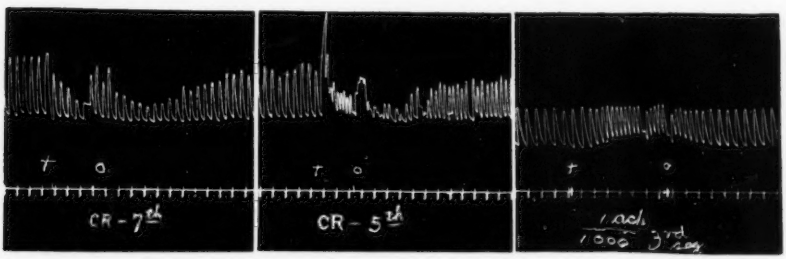

Fig. 6. Determination of pacemaker by localized stimulation with acetylcholine, in the left hand section, a tiny crystal of acetylcholine bromide was placed on the ganglion in the middle of the 7th segment; the depression of contraction suggest inhibition (cf. text). The stimulating effects, however are illustrated in the middle section showing the effects of a crystal of the drug on the ganglion at the 5th segment. A solution of acetylcholine, 1 in 1,000, localized at a capillary point on the ganglion at the 3rd segment likewise stimulated with a shift of the pacemaker function to the minute point stimulated, without augmentation of contractions.

by selection of the concentration of the drug to increase the height of the contraction without affecting the rate. Heinbecker (8) has postulated that, of the two types of ganglion cells originally described by Wm. Patten (10), the more numerous smaller cells are motor cells to the muscle while the larger are capable of initiating the rhythm. Heinbecker presented a diagram of assumed synaptic connections with the smaller cells. Garrey pointed out (4) that it is possible to dislocate the pace-maker function to any minute locus on the ganglion between the third and seventh or eighth segments by punctate warming of the ganglion. It now develops that the same result may be obtained by similarly restricted application of acetylcholine (best in conjunction with eserine). This is easily accomplished by a capillary applicator containing the solution or by soaking a thread in the solution and laying it transversely across the ganglion or by applying a tiny crystal to the ganglion, cf. figure 6. By these means the rate can be accelerated without increasing the height of contractions of the anterior seg-

ments of muscle; in fact, the increased rate may decrease height of contractions as it does in the vertebrate heart. If while the higher rate is thus maintained adequately, stimulating solutions of acetylcholine or lentin be applied along the length of the ganglion there will result a prompt augmentation of the height of contractions. The increased rate can be interpreted only as a direct stimulation of pace-making nerve cells. The augmentation of the muscular response invites further discussion. It has been pointed out previously by Garrey (5) that the prime factor influencing the height of the muscular contraction is the number of motor nerve fibers discharging impulses to the myocardium, and the augmentation here noted is so interpreted, i.e., the increase in height of contraction is due to recruitment. The normal contractions of the heart are never maximal and it is obvious that not all of the motor cells discharge with each beat; there is a large reserve of non-participating, idle cells and augmentation is the result of transformation of these into participating active cells. The evidence is clear that acetylcholine increases the excitability of all the nerve cells, but the additional factor of synaptic facilitation is strongly indicated by the experiments described above and thus we have a dual mechanism by which the motor cells become responsive to the impulses from the pace maker.

Eserine is known to inactivate cholinesterase and it can readily be seen that this action alone would increase the stimulating action of acetylcholine on the ganglion since the ganglion has a high content of esterase which can hydrolyze the esters more rapidly. It is a striking supplement to this action, however, that the potentiating action of eserine, once induced, may be evident several hours later, in fact has been demonstrated in our experiments as much as fifteen hours after a single treatment of the ganglion with eserine. This fact gives rise to certain questions concerning the relation of esterase and ganglionic action. If the ganglionic function is dependent on the action of normally developed acetylcholine, why is it that esterase potentiation by eserine in itself does not lead to accumulation of autogenous acetylcholine and thus cause a progressively increasing activity of the ganglion?[3] This effect has never been noticed in our extensive experience with its use. Again and conversely if esterase is essential to the restoration of normality after each discharge, as has been maintained by Nachmanson, how account for the fact that eserinization of the ganglion by concentrations sufficient to inactivate all esterase does not augment either the height of contraction or the normal rhythmic discharge through the hours during which it can serve as an adequate potentiation agent for acetylcholine? Since the effects of eserine do ultimately wane and disappear, are we to assume a breakdown of an eserine-esterase combination with restoration of the original cholinesterase or are we to assume the development of a new enzyme quantum, and if so, is its origin to be ascribed to neural action? It is to be borne in mind that the ganglion may be dissected free from all other tissue and still maintain its functions by intrinsic action. These questions have yet to be answered before one

[3] F. R. Miller has reported the precipitation by eserine of continuous activity of localized cortical areas of the cat brain resulting in muscular tremors, rigidity and powerful clonus; these effects he attributed to synaptic facilitation.

can be sure that acetylcholine is essential to, or even plays any part in, the normal function of the ganglion.

SUMMARY

1. Acetylcholine, even with the potentiating influence of eserine, is without effect in facilitating the transmission of motor nerve impulses to the musculature of the Limulus heart. This is also true for the cholinesters, lentin and mecholyl and for choline (bromide) itself.

2. Acetylcholine, in physiologically strong solutions (*circa* 1 in 10,000) will stimulate the ganglion, effecting an increase both in rate and amplitude of muscular contractions.

3. Eserine potentiates the stimulating action of acetylcholine on the ganglion thus increasing its effectiveness from twenty to fifty times, an effect which may persist for hours. It does not have this effect with lentin or mecholyl.

4. Atropine stimulates the ganglion. It does not prevent the stimulation by acetylcholine nor the potentiating effect of eserine in combination with acetylcholine.

5. Acetylcholine has a profound effect in restoring function to the depressed (exhausted, fatigued) cardiac ganglion of the Limulus heart. Choline has a similar effect.

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THE EFFECT OF HYPERTONIC PLASMA ON THE BODY FLUIDS IN NORMAL EXPERIMENTAL ANIMALS[1]

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The appreciation of the value of hypertonic plasma in the treatment of shock and other disturbances where an increase in plasma proteins or circulating fluid volume is needed, makes necessary a more comprehensive knowledge of the effects of intravenous injections of hypertonic plasma into normal subjects. The present study is, therefore, an investigation of the changes in body water distribution, electrolytes, and other related factors produced in normal dogs receiving varying amounts of hypertonic plasma.

Freeman and Wallace (1) were able to demonstrate an increase in plasma volume in dogs following the intravenous administration of four times concentrated serum. We (2, 3) have shown that the intravenous administration of concentrated adtevac plasma constantly produces a plasma volume increase in cases of clinical shock and in normal humans. However, the report of Harkins, Boale and Brush (4) is not in complete agreement with these expected results. On the contrary in normal dogs under nembutal anesthesia they demonstrated hemoconcentration following the administration of concentrated plasma.

It is apparent that any change in plasma volume brought about by the intravenous administration of hypertonic plasma is only a part of a generalized shift of body water. The nature and mechanism, as well as the effects of this cellular and extracellular water shift have remained unmentioned and uninvestigated so far.

In this investigation certain complicating factors which deserve special mention made themselves apparent. Reactions due to sodium citrate may be encountered in dogs if 30 to 50 cc. of three or four times concentrated plasma, prepared from citrated blood, is given intravenously. This is, of course, a small dose in adult humans and is entirely without ill effect, but may be attended with temporary reactions in infants and children (2). Thus, the intravenous injection of 80 mgm. of sodium citrate per kilogram of body weight will, in dogs, lead to transient tetany (5) due to the binding of ionic calcium. Vagal paralysis and cardiac standstill (6, 7) may also be produced if sufficient concentration is attained. Reactions consisting of chills and fever (8) are not due to sodium citrate per se (9).

Furthermore, when concentrated plasma prepared from blood collected during

[1] This study was aided in part by a grant from Abbott Laboratories, North Chicago, Illinois.

barbiturate anesthesia[2] is given intravenously, the barbiturate effect becomes a factor. The concentration of the drug in such dog plasma is usually sufficiently low as to produce only mild effects.

Particular efforts were made in these experiments to remove these two complicating factors, but they are considered here in order to emphasize the necessity of taking such factors into consideration in interpreting results of various investigators in the study of the treatment of shock in animals or the effects of administration of concentrated plasma products to normal animals.

The plasma used was first desiccated from the frozen state; consequently denatured protein need not be considered as a complicating factor.

METHODS. Normal healthy dogs were used in these experiments. Anesthesia was not necessary. Medium sized animals were used through preference in order that body water changes might be more readily recognized. The desiccated plasma used was prepared by the adtevac process (10). The concentrated solution was prepared by dissolving the dry product in a reduced volume of distilled water to give an average of 15 grams of protein per 100 cc. Both human and dog plasma were used. Citrated plasma and defibrinated plasma were given in different experiments. These variations are noted in the accompanying table. When citrate and nembutal were present in the plasma they were frequently largely removed by dialysis against saline conducted at low temperatures. After a preliminary period of control studies, dogs were given from 3 to 12 cc. of concentrated plasma per kilogram of body weight. The time of injection varied from one to five minutes. Studies at 15 minute, 30 minute, one hour and various other intervals after the administration of plasma were carried out. These studies included red cell counts using equipment checked by the National Bureau of Standards; hemoglobin by the method of Sanford-Sheard (11) using a Leitz-Mass photoelectric colorimeter; and hematocrit studies using Wintrobe tubes (12). Plasma volume studies were carried out by the blue dye method of Gregersen, Gibson and Stead (13). The injection of human hypertonic plasma containing small amounts of hemoglobin and varying amounts of bilirubin modified the color of the plasma so that plasma volume studies could not always be accurately carried out. In these instances it was felt that plasma volume changes calculated from changes in the hemoglobin concentration were fairly accurate. The extracellular fluid volume, i.e., plasma volume and interstitial fluid volume combined, was calculated as fluid available for solution of sodium thiocyanate (14). Sodium thiocyanate was assumed to disappear at the rate of 0.08 mgm. per 100 cc. per hour (14). Plasma sodium was determined by the method of Butler and Tuthill (15), and potassium by the method of Truszkowski and Zwemer (16). The method of Greenberg (17) was used for the determination of total plasma proteins. All blood samples were collected from the jugular veins.

RESULTS. The accompanying table is a summary of findings in six of the series of eleven dogs. In the remaining five animals identical results were obtained. It will be readily seen that beginning within fifteen minutes after injec-

[2] Nembutal was used in the present study.

tion of hypertonic plasma, hemodilution quickly reached a maximum, as shown by marked and parallel decrease in red blood cell count, hemoglobin, and hematocrit. In accord with this observation, the plasma volume uniformly increased, proportionately in most instances to the calculated hydrophilic property of the injected plasma. The total red cell mass, determined from plasma volume and hematocrit, shows no appreciable change in those animals where dye studies of the plasma volume were satisfactory, so that the plasma volume increase re-

TABLE 1

DOG NO. AND WEIGHT	PLASMA GIVEN	TIME INTERVALS IN MINUTES AFTER CONC. PLASMA INJECTION	RBC IN MILLIONS	HEMOGLOBIN IN GRAMS PER 100 CC.	HEMATOCRIT	MEAN CELL VOLUME	PLASMA VOLUME IN CC.	RBC VOLUME IN CC.	WHOLE BLOOD VOLUME IN CC.	EXTRACELLULAR WATER IN CC.	INTERSTITIAL WATER IN CC.	PLASMA SODIUM IN MGM. / 100 CC.	PLASMA POTASSIUM IN MGM. / 100 CC.	PLASMA PROTEINS GRAMS PER 100 CC.	TOTAL CIRCULATING PROTEINS IN GRAMS	GRAMS OF PROTEIN INJECTED IN PLASMA
No. 1	50 cc.	Control	6.1	13.9			428			2332	1884	343	16	5.1	22	
8.5 kilo	Defib.	20 min.	4.5	11.4			555							5.1		7.5
	Dog	60 min.	4.7	11.4			531			2694	2163	338	16	5.4	28	
		390 min.	5.4										20			
No. 2	30 cc.	Control	7.9	17.6	54	68	562	609	1171	3005	2443	339	20	5.8	33	4.5
9.5 kilo	Defib.	25 min.	6.9	15.6	47	67	600	521	1121					5.9	35	
	Dog	70 min.	6.5	15.4	46	68	620	594	1216	3280	2660	345	19			
		255 min.	7.2	15.5	49	68										
No. 3	70 cc.	Control	7.0	14.3	44	63	469	399	869	2948	2479	336		7.1	33	
9.0 kilo	Defib.	24 min.	5.4	11.9	36	66	644*	399	1043					7.3		10.5
	Human	55 min.	6.0	13.3	41	68	538*	399	937	3033	2495	343				
		300 min.	5.9	12.4	39	67	601*	399	1000	3472	2871			7.1	43	
No. 4	95 cc.	Control	7.0	15.4	48	69	345	362	707	2200	1855	337		6.4	22	
7.5 kilo	Dialyz.	45 min.	5.4	11.4	35	67	520									14.25
	Dog defib.	95 min.	5.7	12.7	41	70	520	360	880	2332	1812	343		6.3	33	
No. 5	90 cc.	Control	5.0	12.0	35	70	780	440	1220	3861	3080	305				13.5
9.2 kilo	Citr.	15 min.	3.5	10.2	29	86	952									
	Dog	60 min.	3.4	10.2	29	86	983	402	1385	4023	3040	320				
		280 min.	4.2	10.8	29	70				4575		288				
No. 6**	30 cc.	Control	6.1	13.4	42	69	433	350	783	2170	1737	352		7.75	34	4.5
7.4 kilo	Citr.	15 min.	5.8	12.0										8.8		
	Dog	75 min.	5.7	13.4	42	73	470							8.4	40	
		160 min.	5.2		39	75	522	326	848	2291	1769	356		8.4		

* Blood volume changes calculated from changes in hemoglobin concentration; all other blood studies by Evans Blue dye method.

** Dog 6 received calcium chloride sufficient to neutralize effect of sodium citrate which was given in the plasma.

flected itself as a similar whole blood volume change. This increase in plasma volume was maintained up to the six hours observed. From the table, it will be seen that there was a slight but distinct increase in mean red cell volume following the intravenous injection of hypertonic plasma. The concentration of sodium thiocyanate in the extracellular fluid (plasma) showed an invariable decrease which was apparent from one to four hours following the injection of concentrated plasma. In the table this finding is interpreted as increase in extracellular fluid.

Changes in sodium and potassium in the plasma were of minor character and principally did not exceed the range of error of the methods used.

The concentration of protein in the plasma showed little appreciable change, the calculation of total circulating plasma proteins revealed an increase roughly corresponding to the amount of injected protein.

In regard to the general effect upon these animals receiving concentrated plasma in proportionately very large amounts, it may be stated that no deleterious results whatever from the plasma itself were observed. In those animals receiving citrated plasma—calculated dose of sodium citrate being at least 80 mgm. per kilogram—temporary tetany occurred from which recovery was quick and complete and was associated with no effect upon the studies of water redistribution carried out as above. In animals receiving non-dialyzed dog plasma, mild nembutal effects, as staggering gait, retching and drowsiness were encountered; here too, the results pertaining to body-water changes were unmodified. No important change in heart rate or respiration was observed. An invariable result of concentrated plasma in normal dogs seems to be defecation and urination.

Discussion. The increase in plasma volume produced in these normal animals by the injection of hypertonic plasma corresponds with most previous experimental studies (1, 2, 3), but seems contrary to the results obtained by Harkins and others (4). This demonstrated effect establishes a firm basis for the use of concentrated plasma in overcoming oligemia of shock, hemorrhage, etc. The indicators of hemodilution (red cell count, hemoglobin concentration and hematocrit) parallel the plasma volume change and demonstrate that storage or release of red blood cells from storage places, such as the spleen, does not influence the results under these conditions. Consequently, a similar study in splenectomized animals seems unnecessary. The degree of plasma volume increase appears to be dependent entirely upon the osmotic effect of hypertonic plasma since the amount of fluid entering the blood vessels is proportional to the total amount of protein injected.

The two major possible sources of this increased plasma fluid are the interstitial and intracellular water compartments, although fluid in the lumen of the gastrointestinal tract must also be considered.

Various factors influence the shift of water across membranes from one compartment to another. Body cells act as osmotic units, susceptible to changes in the osmotic pressure of their environment, the interstitial fluid (18). If the osmotic pressure in the interstitial fluid be increased, water diffuses out of the cell into the extracellular spaces (18, 19). The converse causes water to diffuse into the cell.

When the plasma osmotic pressure is increased a series of events seems to take place. The processes of osmosis and diffusion rapidly transmit the increased osmotic pressure to the interstitial compartment by the passage of fluid from the interstitial space to the plasma. At the same time ions diffusible through the endothelium pass from plasma to the interstitial water. These mechanisms reëstablish the osmotic equilibrium between plasma and interstitial water re-

sulting in a final increased osmotic pressure in both compartments of the extracellular fluid. In order to reëstablish osmotic equilibrium between the cells and their environment, fluid then passes from the cell into the extracellular space, increasing the interstitial fluid volume.

We have attempted to measure these variations in the volume of extracellular fluid by changes in the amount of fluid (extracellular) available for solution of thiocyanate and also by changes in the concentration of sodium in the extracellular fluid (since sodium is an ion which is non-diffusible through the cell membrane). The invariable dilution of thiocyanate in one to four hours after the injection of hypertonic plasma seems to be strong qualitative evidence for the occurrence of such an increase of extracellular fluid as postulated above on the basis of body fluid dynamics.

In some experiments there was somewhat greater increase of extracellular water than that expected from the protein concentration alone of the solution injected. Since the hydrophilic effect of protein is dependent on changes in hydrogen ion concentration and the concentration of other cations, the question may not be completely answered by osmotic forces.

The failure of sodium to decrease with this increased extracellular water is probably explained by the additional amount of sodium injected, this being proportional to the increased water in the extracellular compartment.

In view of the findings here reported it becomes apparent that the interstitial and intracellular water compartments constitute large available reservoirs of water for supplementing plasma fluids. Furthermore, the mobility of cellular and extracellular water in this sense takes on added significance in considering the disturbances of body water distribution in shock, hemorrhage, burns, infections with parenchymatous degeneration (intracellular edema) and in the treatment of these conditions.

Although in some instances as in experimental hypertonic saline shock and in hemorrhagic shock with anoxemia (19), the changes in mean red cell volume corresponds with tissue cellular fluid, in these experiments it is apparent that no such correlation exists.

SUMMARY

1. Intravenous injection of hypertonic plasma in normal dogs leads regularly to a long sustained increase in plasma volume more or less proportional to the amount of protein injected, the red blood cell count, hemoglobin, and hematocrit decrease indicating a proportional hemodilution. No appreciable changes in size of the red blood cells were produced. The total circulating mass of red blood cells does not seem to be altered.

2. The total extracellular water volume is increased after the injection of concentrated plasma as shown by dilution of thiocyanate. The source of this water is assumed to be mainly from cellular water and the redistribution would appear to depend upon principles of osmosis and diffusion between the cells as osmotic units and their surrounding fluid environment.

3. The availability of cellular water in this manner is considered to be of sig-

nificance in the correction of oligemic states. Also the mobility of cellular water suggests further defects occurring in shock, hemorrhage, burns, infections, etc., and indicates a possible therapeutic attack of such a body water defect.

4. In considering the presumed ill-effects of concentrated plasma in small animals, it should be kept in mind that certain extrinsic substances such as sodium citrate, nembutal, denatured proteins, allergens, etc., may be present in sufficient quantity to produce observable effects. This would seem to be of importance in appraising results in the treatment of experimental shock.

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COMBINATION OF HYPOXIC AND HYPERCAPNIC STIMULATION AT THE CAROTID BODY

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It is common experience to witness under various circumstances the mutual facilitation of the two common respiratory excitants, carbon dioxide excess and oxygen lack. Recently Dill and Zamcheck (1940) demonstrated quantitatively their additive effects in man, and Gellhorn and Lambert (1939) reported evidence from narcotized dogs of their synergistic effects on blood pressure.

The latter authors also raised anew the question concerning the site(s) or mechanism(s) of mutual facilitation of the two excitants. Their experiments allowed the obviously probable interaction of centrally impinging afferent impulses from the carotid and aortic bodies, with direct central changes caused by CO_2-excess and O_2-want. Gesell, Lapides and Levin (1940) demonstrated the interaction of these "reflexogenic" and "centrogenic" components during eupnea, hypercapnia and anoxemia.

In addition Gellhorn and Lambert interpreted results of their experiments on chemoceptively-deafferented dogs to indicate direct synergistic interaction of hypercapnia and hypoxemia at the vasomotor "center", and reinterpreted results of Selladurai and Wright (1932) and Smyth (1937) to allow of the same for the respiratory center. Dumke, Schmidt and Chiodi (1941), however, do not agree. Although a *potentiation* of the CO_2 response by hypoxemia was apparent after denervation in 5 of their 14 animals, and examination of their mean data for the 14 animals shows that the experienced effect of a combination of hypoxemia and hypercapnia approximated the algebraic addition of the individual separate effects,[1] they draw, it would seem, an unwarranted conclusion that "the additive effects of anoxemia and hypercapnia on respiratory minute volume" in intact animals "were therefore due entirely to chemoreceptor reflexes aroused by the former". This statement, of course, would exclude not only direct addition at

[1] The mean minute-volume of respiration was:

During O_2-administration	4720 cc.
During hypoxemia	3470 cc. (−1250 cc.)
During hypercapnia (with high O_2)	8040 cc. (+3320 cc.)
During combined hypoxemia and hypercapnia	6580 cc.
Calculated combination effect, on the basis of algebraic addition (4720 − 1250 + 3320)	6790 cc.

The actual minute-volume during combination of conditions thus deviated from that predicted by algebraic addition of the separate effects by 3 per cent; the *change* in minute-volume (from basal), by 10 per cent.

the "center" which their own data suggest, but also a third possible point of addition—at the peripheral chemoreceptors.

There is now no doubt that both hypercapnia and hypoxemia are capable of chemoreflex excitation of respiration through the carotid bodies. Differences of opinion exist concerning thresholds and potency. Though available evidence indicates that this reflexogenic support of breathing is more important in hypoxia than in hypercapnia, there appear to be no actual data on its contribution during a *combination* of these conditions (asphyxia). So long as the chemoreceptors retain some possible importance in the regulation of breathing in the intact organism, either in eupnea or stress, or both, it seems of interest to report the following data, evidencing a mechanism for *local* addition within them of hypoxic and hypercapnic chemoreflex effects.

The apparatus and methods used are described in more detail elsewhere (Winder, 1937a, b). The vascularly isolated carotid segments were perfused with a heparinized mixture of 52 to 77 per cent defibrinated blood in Locke's solution (except expt. 5, in which 35 per cent whole blood was used), through the common carotid arteries as inlets and lingual arteries as outlets, at approximately mean arterial pressure or somewhat above, by means of a continuous artificial circuit which continuously equilibrated the basal perfusing fluid with a gas mixture containing between 5.95 and 6.06 per cent CO_2 (in the various experiments), and over 93 per cent O_2. Accessory reservoirs, each connected with a collapsible bag containing the proper gas mixture, were used for shunting anoxic, hypercapnic or "asphyxial" fluids into the circuit beyond the aerator, without change in perfusion pressure. The anoxic fluid was previously equilibrated with a gas containing less than 0.5 per cent O_2 and the same CO_2 content as the basal mixture (the difference ranged from 0.07 per cent less to 0.12 per cent more; average, 0.016 per cent less); the hypercapnic fluid with 35 per cent CO_2 and 64 $\pm$ 1 per cent O_2; the "asphyxial" fluid with 35 per cent CO_2 and less that 0.5 per cent O_2. Temperature in the circuit was controlled electrically.

Mean (femoral) arterial pressure and perfusion pressure were recorded with mercury manometers, and respiration with a recording spirometer attached to a rebreathing circuit. The vagus nerves were intact.

As indicated in the figures, the experimental fluids were administered for short periods of 25 to 46 seconds (average, 32 sec.). There was considerable volume in the circuit between the point of admittance and the carotid, so that during the whole of such short periods the experimental fluid was undoubtedly diluted with basal fluid before reaching the carotids. That is, the effective potency of the experimental fluids was considerably less than would be implied by the gas mixtures used. However, in any one experiment reported, circumstances were unaltered while the three fluids were being used, so that the responses are comparable with one another with one qualification: Because these procedures were originally intended in another connection, the durations of administration were unfortunately not equal in any one series. Consequently, in each experiment *only the first parts of the responses are quantitatively comparable.* Specifically, for

a given experiment the responses are deemed comparable up through a period equal to the sum of the latent period and the shortest administration period among the three trials; i.e., up to the right borders of the vertical rectangles in the figures. (*Vida* "latent period" and "duration of briefest administration".) The "basal respiration" in each experiment is the mean of points before the beginnings of administrations. The "calculated combination" response is the algebraic addition of the separate CO_2 and low O_2 responses, with reference to the basal respiration. The sequence of procedure varied, with the "asphyxial" fluid intermediate in order in experiments 2, 3 and 4.

All the pertinent data, from the five experiments applicable to the present problem, are reported in the corresponding figures. *The addition of the reflex respiratory effects of hypoxia and hypercapnia in combination at the carotid bodies is clear in experiments 1 to 4.* The effect of the actual combination (asphyxial fluid) agrees closely with the calculated effect of the combination of CO_2 and low O_2. There were, of course, uncontrollable irregularities such as occasional deep breaths. Either alone or in combination, hypoxia no doubt altered CO_2 transport, while hypercapnia altered oxygen transport and oxidations; but such factors in the end only contributed to the total of added CO_2 and low O_2 effects.

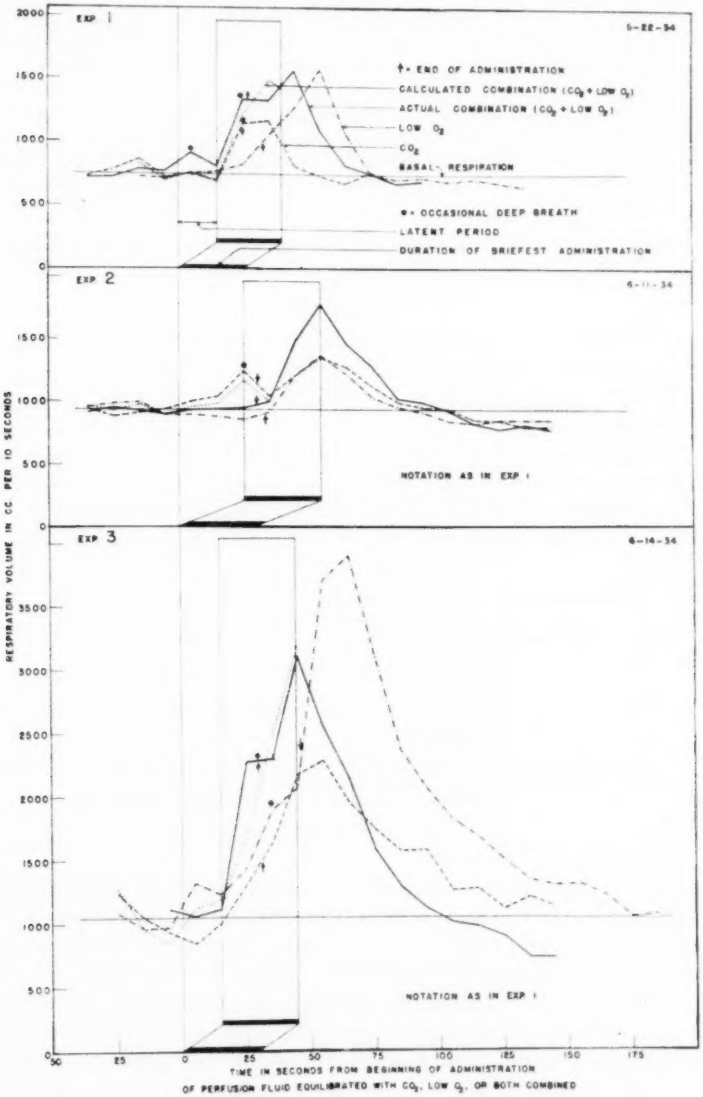
It is recalled that the perfusion fluid in experiment 5 contained least blood, and that in this case alone the blood was not defibrinated. At its face value, the outcome of experiment 5 would appear to be an exception, in that all three procedures yielded approximately the same responses, qualitatively and quantitatively. This fact in itself, however, in the face of differentiation of responses in all the other experiments, probably indicates a *low ceiling* of response of an accidentally poor preparation. This interpretation is further supported by the fact that the maximal responses of all other preparations to the same or more brief administrations considerably exceeded the responses of this one in steepness and extent. Thus, *rather than providing a true exception, the results of experiment 5 apparently express accidental circumstances masking the normal addition of hypoxic and hypercapnic effects.*

It should be pointed out that in these brief administrations the responses were cut short of their maximal. It is evident that they were increasing, usually steeply, at the ends of the comparable periods. That is, the data reported here actually pertain to the summated contributions of hypoxic and hypercapnic stimulation to the *onset* of carotid body excitation. It is probable, of course, that summation in onset is continued in the steady state. Further, these brief procedures offer the probable advantage of providing responses least complicated by secondary changes in the organism, such as hypocapnia.

Concerning the fundamental mechanism through which this summation of stimuli operates, the facts at present either strongly support or satisfy the hypothesis that intracellular acidity, representing interaction between metabolism and immediate environment, is an important circumstance in excitation of carotid body chemoreceptors.

Their internal structure and rich sinusoidal circulation predict an active metabolism (De Castro, 1926, 1927–28). During local chemical suppression of anaerobic glycolysis

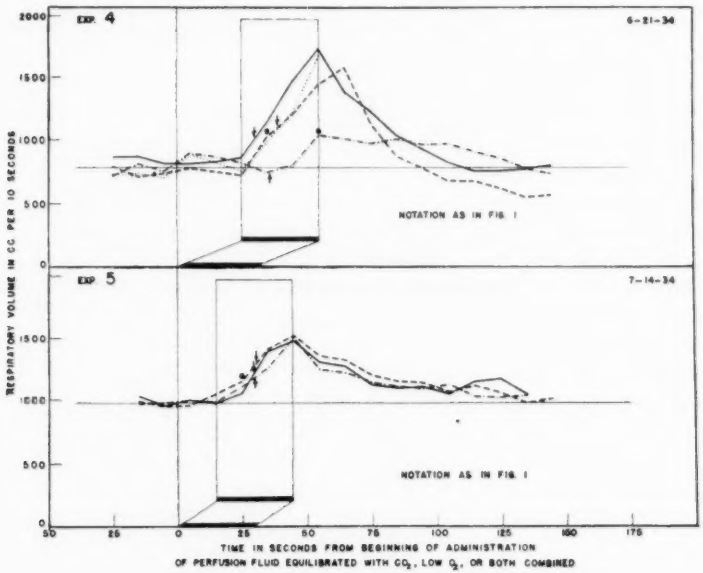
their normally strong hypoxic excitation is replaced by depression while their hypercapnic excitation may persist (Winder, 1937b). Local thermal (Bernthal and Weeks, 1938;



Figs. 1-3

Schmidt, Comroe and Dripps, 1939) or chemical (Shen and Hauss, 1939) increase in metabolism and acidity increases their excitation. During ischemia, cumulative interaction between their metabolism and a static environment results in a strong excitation (Winder,

1938a; Winder, Bernthal and Weeks, 1938; cf. also Bogue and Stella, 1935; Samaan and Stella, 1935; Stella, 1936; Rudberg, 1938, 1940). Their degree of excitation in general parallels their theoretical internal acidity under a variety of chemical conditions (Bernthal, 1938; von Euler, Liljestrand and Zotterman, 1939), and they respond delicately to *exogenous* acidity (Bernthal, 1938; Schmidt, Comroe and Dripps, 1939). Cell cH is very probably not the *only* fundamental factor involved in ultimate stimulation of the receptors. For example, four years ago, in view of the potency of acetylcholine in exciting the carotid body (Winder, 1938b; unpublished experiments indicate that eserinization increases its potency to approximately that of nicotine), we proposed that this or a similarly acting material, liberated and protected by metabolic acidity, may possibly be involved in normal transmission of excitation from the epithelioid cells to their contiguous nerve endings (Winder, 1938c; Gesell, 1939, footnote 1).



Figs. 4 and 5

Attention is directed once more to the lack of necessary parallelism between blood cH on the one side and probable internal acidity and receptor activity on the other (cf. Bernthal, 1938). In the present experiments, of the three experimental fluids the "asphyxial" must have had an intermediate cH, yets its influence on receptor activity and probably cell cH was greatest. Until this application of the "reaction" theory (Gesell, 1925, 1929) to the chemoreceptors fails to meet its requirements, we may ascribe the addition of hypoxic and hypercapnic excitation at the carotid body to changes associated with convergence of their respective acid mechanisms probably upon common receptor cells (Winder, 1937b; von Euler, Liljestrand and Zotterman, 1939).

The present data extend those of Bernthal (1938), von Euler *et al.* (1939), Gesell *et al.* (1940) and others, which indicate that *tonic* activity of the carotid

bodies (in anesthetized animals) during eupnea is contributed to by the combined effects of resting O_2 and CO_2 tensions in the blood. They indicate that in the combination of hypoxic and hypercapnic respiratory effects in the organism as a whole, one must now consider *first*, direct combination at the carotid body; *second*, a probable direct combination centrally as affirmed by Gellhorn and Lambert (1939) and allowed by the data though not the conclusion of Dumke *et al.* (1941); and *third*, indirect combination through interaction at the "center" of the *pre-combined* reflexogenic and centrogenic influences (cf. Gesell *et al.*, 1940). It must be recognized that particularly in the more intricate nervous centers, the local combination includes potential depression concurrent with potential excitation (Gesell, 1939; Winder and Winder, 1941; Moyer and Beecher, 1941).

SUMMARY

The isolated carotid body regions of anesthetized dogs were perfused by means of a continuous artificial circuit with continuous gaseous equilibration of the basal perfusion fluid (heparinized blood and Locke's solution). Brief substitutions of experimental fluids equilibrated with gases high in CO_2, low in O_2, or a combination of the two, yielded reflex respiratory responses such that the combination effect on minute-volume of CO_2 and low O_2 was approximately equal to the sum of their individual separate effects.

The results are interpreted in terms of the theory that intracellular cH is a factor in the control of chemoreceptor activity.

The chemoreceptors are considered as one of several probable sites for mutual facilitation of hypoxic and hypercapnic stimulation of respiration.

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THE FLOW OF LYMPH FROM THE LUNGS OF THE DOG

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If one uses the conventional reasoning relative to exchanges between the blood and tissue fluids, the lungs of mammals apparently have little need for a system of lymphatic vessels. The pressure in the pulmonary capillaries has never been measured directly, but it should be low in view of the extent of the capillary bed and the low pressure in the pulmonary artery. At the same time the blood in the lungs has the colloid osmotic pressure of the blood throughout the body, so that a relatively dry tissue should result. In the face of these considerations, which seem to make an extensive system of lung lymphatics unnecessary unless under the stress of pulmonary infection or cardiac incompetence, there stands the fact that the lungs are profusely provided with lymphatics.

Cunningham (1916) showed that the capillary lymphatics of the mammalian lung do not extend beyond the alveolar atria and Miller (1937) found no lymphatics beyond the alveolar ducts. These findings mean that proteinized fluid in the alveolar walls or actually within the alveoli does not enter the lymphatic system until the beginning of the respiratory part of the lung lobule is reached. The lymphatics of the lungs can be divided into a superficial and a deep set. The former is situated in the pulmonary pleura; the latter accompanies the bronchi, the pulmonary arteries, and the pulmonary veins. It also forms a dense network in the connective tissue septa between the secondary lobules. The two sets of lymphatics are in communication within the pleura and at the hilum of the lung. The pleural lymphatics eventually unite to form trunks which, together with the lymphatics coming from the interior of the lung along the pulmonary veins, enter the tracheobronchial lymph nodes (Miller, 1937). Wherever lymph is formed it flows eventually to nodes at the root of the lung or along the trachea.

Sappey (1874) declared that in mammals most of the lymph from the right lung entered the venous system through the right lymphatic duct, whereas the thoracic duct received lymph from the left lung. Variations on the left side were common and occasionally a vessel entered the junction of the jugular and subclavian veins quite independently of the thoracic duct.

In man, Rouvière (1932) reported that lymph from the superior and upper middle part of the left upper lobe entered the left tracheobronchial chain of nodes and lymphatics to reach the thoracic duct or the junction of the left subclavian and left jugular veins. The rest of the lymph from the left lung, together with some from the lower right lobes enters the nodes at the bifurcation

of the trachea. Efferent lymphatics from this group of nodes practically always pass to the right tracheobronchial nodes, rarely to the left. These nodes also receive lymph from the upper lobes of the right lung. The major part of the lung lymph enters the venous system at the junction of the right jugular and right subclavian veins, either directly or in conjunction with the right lymphatic duct.

In the dog Baum (1918) found that lymph from both lower lobes entered the medial tracheobronchial lymph node. His efforts to trace the lymph flow from other lobes and the possible communications between the right and left lung lymphatics either directly or through nodes are not clear.

In 1931, being under the impression that lymph from the heart and lungs entered the thoracic duct, we made Starling heart-lung preparations in dogs, cannulated the duct at the entrance into the left subclavian vein and ligated it just above the diaphragm. Under these circumstances we believed that all the lymph which might be obtained from the thoracic duct must come from the heart and lungs. No effort was made to cannulate or even observe the right lymphatic duct. In experiments—wholly satisfactory from a technical point of view—no lymph or, at the best, a few drops were collected from the thoracic duct. Either little or no lymph was formed in the chest or another path carried it to the blood.

Later, through a posterolateral incision, lymphatics were cannulated at the root of the right lung. These vessels were filled with lymph but it was always grossly bloody, due undoubtedly to lung trauma incident upon the method of exposure. The experiments did show that lymph flowed freely from the lungs if they had been rendered abnormal.

Lorber (1940) used a Starling heart-lung preparation in dogs, again with the thoracic duct tied just above the diaphragm and cannulated at the entrance into the subclavian vein. In accord with our experience he found no increase in lymph flow in spite of the development of massive pulmonary edema.

During a long period of work upon the flow and composition of lymph from the heart of the dog (Drinker et al., 1940), several lymphatics were seen connected with a lymph node under the superior vena cava and along the right side of the trachea. This node is also in the line of cardiac lymph flow, but with experience, particularly that gained from injections into the heart muscle, cardiac vessels can be distinguished from the remaining lymphatics entering the node which carry lymph from the lungs. The facts relative to the lymph collected, with proof as to lines of drainage from the lungs, form the substance of this paper.

EXPERIMENTAL PROCEDURE AND RESULTS. Healthy, young, adult dogs of medium size, anesthetized with nembutal (40 mgm. per kgm. intravenously) were used. Several cats were examined but the lymphatics were too small to cannulate successfully. It is impossible to reach the lung lymphatics without opening the chest, so that the trachea was cannulated and artificial respiration given by a pump delivering constant inspiratory volumes of air or oxygen with expiration following passively—that is, without suction.

The thorax was opened by removal of a section of sternum plus 3 to 5 ribs,

depending upon the extent of the exposure required. The ribs were cut about 3 cm. from their sternal connections. Both mammary arteries were tied and divided, and the thymus was removed if present in any size. Beneath the superior vena cava, on the right side of the trachea, 5 to 7 cm. from the bifurcation, one finds the tracheobronchial lymph node which receives lymph from both the heart and lungs. This node was pulled gently upward and to the right and a vessel entering upon the lower side was cleaned and isolated. It is invariably the largest of several entering the node from the lungs. This vessel was tied and cannulated with a small pyrex glass cannula. Care was taken to support the cannula in such a way that the tip lay in the line of normal drainage. The lymphatic selected is larger than the cardiac lymphatic and, being further from the heart, the motion at the point of cannulation was not so disturbing. A fine wire with a small loop at the end, dipped in dry heparin, was placed in the cannula to prevent the lymph flowing into it from clotting. The vessel cannulated is not the entire pathway for lymph from the lungs. Other and variably placed lymphatics exist and the results gained from this single trunk are thus indicative of general trends but do not give quantitative results for the total pulmonary lymph flow.

Early in the experiment, 20 cc. of Ringer's solution per kilogram of body weight were given intravenously to insure a sufficient amount of fluid in the tissues. Systemic blood pressure was taken from the femoral artery with a mercury manometer. Pulmonary arterial pressures were taken with a second mercury manometer connected to a stilette cannula in the pulmonary artery (Swift et al., 1922).

It is probable that lymph flow in the unopened chest is somewhat greater than that obtained in our experiments. The preparation, admitting the disadvantage of artificial respiration is, however, far less abnormal than the usual open chest experiment, in that the exposure through the anterior mediastinum is so small as to permit one to see only the tips of the lungs and the base of the heart within the pericardium. There is thus little drying, etc., factors so prominent in most open-chest experiments and obviously detrimental in an effort to secure lymph from normal lungs. Rate of flow was measured by collecting lymph for a given period of time into weighed tubes which were then reweighed. Protein determinations were made by means of the Zeiss dipping refractometer calibrated against known samples of lymph.

A. *The composition of lung lymph.* Table 1 shows the protein concentration, in per cent, of lung lymph collected from 18 dogs. The average amount of protein was 3.66 per cent, with a range from 2.81 to 4.65 per cent. In protein content, lung lymph is very much like cardiac lymph. It contains albumin and globulin in the ratio found in blood and in lymph from other regions. It also contains fibrinogen and clots. Erythrocyte and leucocyte counts are included in table 1. The relatively large number of red cells in the lymph from the early experiments is ascribable to slower and less efficient operating. It became less as technique improved. The white cells, practically all lymphocytes, are due to the passage of the lymph through several nodes before reaching the point of cannulation.

B. *Conditions affecting the flow of lung lymph.* 1. *The normal flow of lung lymph.* Figure 1 is a typical experiment showing the normal flow of lung lymph from a single vessel over a period of several hours. Lymph from the lungs, which are in constant motion, flows steadily under normal conditions. Table 1 shows the average lymph flow in a series of 18 dogs. This, as has been pointed out, does not represent the total amount of lymph produced by the lungs, but the amount obtained from one of several efferent lung lymphatics. The average amount of lymph from this single lymphatic was 17.9 mgm. per minute, with a wide range of from 3.7 to 59.4 mgm. per minute.

2. *Increased pulmonary ventilation.* Artificial respiration was provided by a pump calibrated to deliver a given amount of air in 17 inspirations per minute.

TABLE 1

Protein concentration, lymph flow, and cell content of lung lymph from 18 dogs

NUMBER OF EXPERIMENT	PROTEIN	AVERAGE LYMPH FLOW PER MINUTE	ERYTHROCYTES PER CM.	LEUCOCYTES (LYMPHOCYTES) PER CM.
	per cent	*mgm.*		
1	3.38	23.1		
2	3.09	14.6	22,350	23,850
3	3.71	22.7	13,850	11,900
4	3.87	6.8	20,100	8,850
5	3.77	6.5		
6	4.00	3.7		
7	3.76	3.9	6,800	2,900
8	4.10	5.1	30,700	28,100
9	3.05	27.8	17,900	16,300
10	4.00	4.6	100	100
11	3.63	11.2	15,800	15,000
12	3.75	7.1	7,600	20,500
13	3.48	4.5	900	22,800
14	2.81	10.3	3,900	44,600
15	3.26	37.7	200	26,600
16	3.08	59.4	1,500	13,700
17	4.46	41.9	300	76,400
18	4.65	30.6	0	21,500
Average	3.66	17.9	9,533	22,206

As soon as the chest was opened the pump was so adjusted as to provide adequate ventilation, usually from 2.5 to 3.25 liters per minute, depending upon the size of the animal. Figure 2 shows one of a group of experiments in which, during the control period, while lymph was being collected, the animal was receiving 2.5 liters of air per minute. Between the vertical lines 1 and 3, pulmonary ventilation was increased to 4.3 liters per minute. The lymph flow immediately decreased and remained constant until the ventilation was reduced to its original volume. The flow then rose again to its control level.

The fall in lymph flow with increased artificial respiration is probably not what occurs when there is a normal increase in breathing. There is evidence that heightened negative pressure in the thorax causes transudation into the

pleural sacs. Graham (1921) held that in the presence of pulmonary edema, deep respiratory movements, with consequent increased degrees of negative intrathoracic pressure, sucked fluid from the lungs into the pleural sacs. Brock and Blair (1931), using a heart-lung preparation, showed the development of pleural transudates just as Graham had postulated. Yamada (1933) made pleural punctures in several hundred healthy soldiers. He obtained pleural fluid in 29 per cent of his subjects who had been entirely quiet. Seventy per cent of the same subjects yielded fluid after a period of severe work. This means that negative pressures of 30 to 40 mm. Hg, such as occur readily during work when inspiration is full, with a return to 6 mm. or thereabouts at the end of expiration, cause fluid to leak from the surface of the lungs. The effect of the change in pressure on the volume of the lung capillaries is thought to be slight. But if a transudate can be made to accumulate in the pleural sacs as a result of

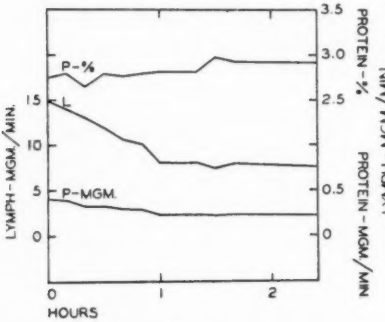

Fig. 1

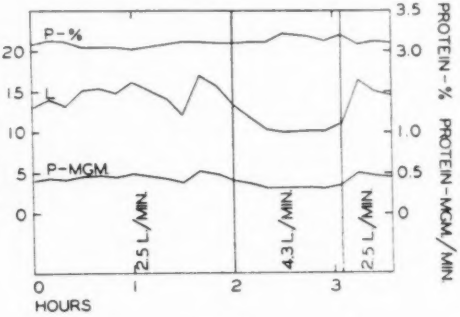

Fig. 2

Fig. 1. Normal flow and composition of lung lymph. *Upper curve*, protein in lung lymph in per cent. *Middle curve*, milligrams of lymph per minute. *Lower curve*, lymph protein in milligrams per minute. *Ordinates*, as designated. *Abscissae*, time in hours.

Fig. 2. Effect of over-ventilation. *Upper curve*, protein in lung lymph in per cent. *Middle curve*, milligrams of lymph per minute. *Lower curve*, lymph protein in milligrams per minute. *Ordinates*, as designated. *Abscissae*, time in hours.

hard exercise, it would seem that accumulation of fluid in the lung tissue must occur at the same time and that the high negative pressure at full inspiration may be part of the cause. That the lymphatics would take part in the removal of this fluid is reasonably certain.

With the chest open and with artificial respiration, pressure upon the lung capillaries is always positive and when respiration is increased by altering the stroke of the respiration pump, but not the rate—as was done in these experiments—a relatively high positive pressure will be present through most of the respiratory cycle. The result will be the maintenance of tissue pressure in the lungs upon a positive level rather than the customary negative pressure and a tendency to reduce transudation of fluid rather than to increase it.

3. *Continuous intratracheal insufflation.* After a control period, during which artificial respiration with pure oxygen was given by means of the respiratory

pump, the animal was shifted to intratracheal insufflation with pure oxygen, lung inflation being held just below the complete inspiratory position. Figure 3 shows a typical experiment in this series. With no lung movement at all—arrows *1* and *2*—and only the massage caused by the movements of the heart and pulmonary vessels, the flow of lymph fell abruptly, but did not stop entirely. As soon as the intratracheal tube was removed and artificial respiration again supplied by the pump, the flow of lymph returned to approximately the control level. In this experiment, cessation of lung movement, with the lungs not ab-

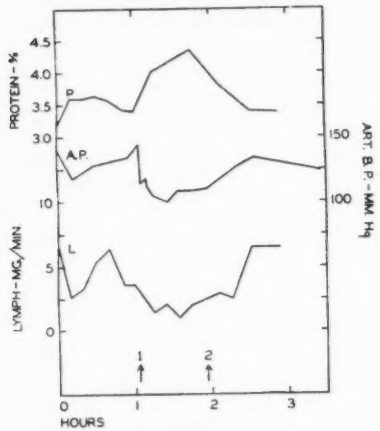

Fig. 3

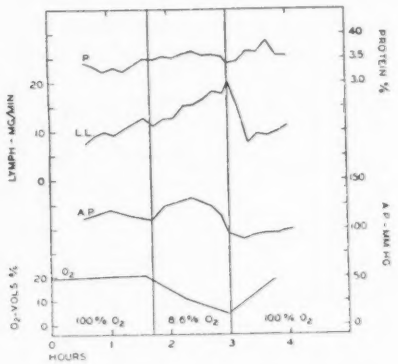

Fig. 4

Fig. 3. Effect of holding lungs motionless. *Upper curve*, protein in lung lymph in per cent. *Middle curve*, pressure in the femoral artery in millimeters of mercury. *Lower curve*, milligrams of lymph per minute. *Ordinates*, as designated. *Abscissae*, time in hours. Between arrows *1* and *2* rhythmical artificial respiration ceased and continuous intratracheal insufflation was employed. At arrow *2* rhythmical artificial respiration was again initiated.

Fig. 4. Effect of lowering alveolar oxygen. *Upper curve*, *P*, protein in lung lymph in per cent. *Second curve*, *L.L.*, milligrams of lymph per minute. *Third curve*, *A.P.*, pressure in the femoral artery in millimeters of mercury. *Fourth and lowest curve*, O_2, cubic centimeters of oxygen per 100 cc. of arterial blood. *Ordinates*, as designated. *Abscissae*, time in hours. Between the vertical lines the animal was given 8.6 per cent O_2 and 91.4 per cent N instead of 100 per cent oxygen. Rate and stroke of respiratory pump constant throughout the experiment.

normally distended, resulted in a fall of systemic blood pressure but not to a serious degree and certainly not enough to change the pulmonary blood pressure significantly. Lymph flow was less than in the moving lung and reached a high figure after rhythmical artificial respiration was restored. The degree to which continuously insufflated oxygen reaches all parts of the lungs is problematical and the possible concomitant effects of oxygen lack in parts of the lungs can not be excluded. But even if abnormal amounts of tissue fluid or lymph have been formed during the period of quiescence, it is apparent that respiratory movements are important for causing lymph flow.

4. *Reduced oxygen.* Figure 4 shows one of a series of experiments in which, after a control period of ventilation with 100 per cent oxygen, the animal was given a mixture of 8.6 per cent oxygen and 91.4 per cent nitrogen by the respiration pump for 73 minutes. When inspiratory movements which often accompany inadequate oxygenation occurred they were controlled by intravenous injections of 1 cc. of 1 per cent curare, diluted to 10 cc. with Ringer's solution. Femoral arterial pressures were recorded throughout and the oxygen content of the arterial blood was determined frequently. As soon as the animal began to receive the low oxygen mixture, the flow of lymph from the lungs increased. The femoral blood pressure fell after the initial rise which sometimes attends abrupt anoxemia. When the arterial blood pressure fell to about 95 mm. of mercury and the oxygen saturation of the arterial blood was 4.42 volumes per cent, the ventilation of the animal was shifted back to pure oxygen. Lymph flow returned almost immediately to the normal level. That the period of severe oxygen lack did no permanent damage to the blood capillaries is shown by the fact that at the end of the experiment, when the oxygen content of the blood had

TABLE 2

Oxygen and carbon dioxide in the arterial blood during ventilation with 100 per cent oxygen, low oxygen, and return to the original condition

TIME	OXYGEN PER 100 CC. BLOOD	CARBON DIOXIDE PER 100 CC. BLOOD	REMARKS
minutes	*cc.*	*cc.*	
0	19.31	29.44	Changed from pure oxygen to 8.6 oxygen at 107 minutes. Returned to 100 per cent oxygen at 180 minutes
95	20.58	28.95	
135	10.86	31.7	
178	4.42	14.5	
225	19.04	29.98	

returned to the control level of 19 vols. per cent, there was no increase in the amount of lymph and no appreciable increase in protein content.

Table 2 gives the oxygen and carbon dioxide content in cubic centimeters per 100 cc. of blood during the experiment. In this animal blood oxygen was at a normal level whenever 100 per cent oxygen was used for ventilation, but the carbon dioxide was abnormally low throughout the entire experiment and fell markedly during the period when 8.6 per cent oxygen was supplied. In other experiments where the carbon dioxide content of the arterial blood was 39 to 43 cc. and the oxygen content fell markedly on ventilation with low oxygen, lymph flow increased as in this case, so that low oxygen alone causes an increase in the permeability of the lung capillaries. The relation of this effect to the carbon dioxide content and combining power of the blood, particularly to the situation provided by holding the blood carbon dioxide above normal during ventilation with low oxygen mixtures are not known. Maurer (1910) has shown that in the presence of a normal oxygen content, increased carbon dioxide in the blood caused the capillaries of the heart and of the skin, mucous membranes,

etc., in the head of the dog to become more permeable as judged by the outflow of lymph, but the result was never as marked as that obtained from uncomplicated anoxemia.

5. *Increased pulmonary blood pressure.* The effect of increasing pulmonary blood pressure on the flow of lymph from the lungs was observed in a number of dogs. Femoral arterial and pulmonary arterial pressures were recorded with mercury manometers. A stilette cannula, as described by Swift, Haggart and Drinker (1922), which is thrust through the wall of the pulmonary artery, was used for the pulmonary blood pressure measurements since it causes no obstruction to pulmonary blood flow and can be inserted without manipulation of the pulmonary vessels. After the pericardium was incised longitudinally and stitched to the sides of the opening in the chest, a ligature was passed around the pulmonary veins and attached to a screw clamp which could be so adjusted as to produce and maintain any desired degree of vein compression (Drinker, Peabody

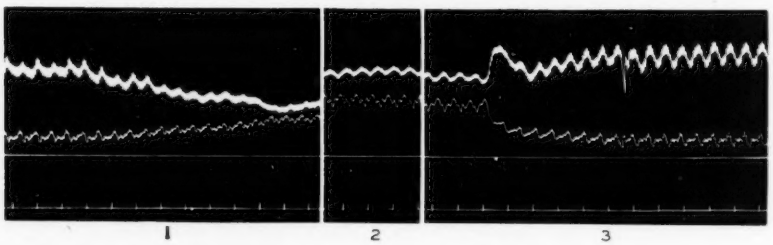

Fig. 5. Effect of compressing the pulmonary veins. Tracings of systemic (*upper curve*) and pulmonary artery (*lower curve*) pressures during 1, tightening; 2, maintenance of steady compression, and 3, release of the compression clamp on the pulmonary veins. *Straight line*, base for pulmonary blood pressure; *bottom line*, time in 6-second intervals and base for systemic pressure. Tightening of the compression clamp began during tracing *1*, was held steady as shown in tracing *2*, and was released in the early part of tracing *3*.

and Blumgart, 1922). This arrangement obstructs the flow of blood in the pulmonary veins just as they reach the left ventricle, but does not affect other vessels entering and leaving the heart. In order to put the clamp in place, a longer opening must be made in the chest, but the exposure required is essentially at the base of the heart and even in these experiments was less extensive than in many direct examinations of the heart and lungs.

Lymph flow was measured during a control period. The pulmonary arterial cannula was then inserted and connected to the manometer and, while recording pulmonary and systemic pressures, the pulmonary veins were compressed by tightening the clamp. Figure 5 shows kymographic records of the systemic and pulmonary artery pressures in a typical experiment. The upper curve is that of pressure in the femoral artery, that just beneath it of pressure in the pulmonary artery. Tightening of the compression clamp upon the pulmonary veins began during segment 1 of the tracing, and caused a simultaneous fall in systemic and rise in pulmonary artery pressures. The clamp was closed slowly until a point

was reached where systemic and pulmonary arterial pressures were stabilized at new levels, as shown in segment 2 of the tracing. The sequence of events in such an experiment is as follows: As the pulmonary veins were slowly compressed, blood flowed steadily into the right side of the heart from the cavae and was pumped into the pulmonary circulation where, on account of obstructed flow, it formed an enlarging pool. After a time, venous occlusion being moderate in extent, the pulmonary vascular bed was stuffed full and blood began to move over into the left side of the heart in fair volume so that cardiac output improved and systemic blood pressure rose. At the same time, pressure in the pulmonary artery, due to a competent right ventricle, was held at a high level. In segment 3 of the tracing, the double mark upon the base line for the pulmonary arterial pressure signalled the point at which the pulmonary vein clamp was abruptly released. There was an immediate rise in the systemic blood pressure as the blood imprisoned in the lungs flowed rapidly into the left side of the heart. The pulmonary arterial pressure fell to the original level.

That part of the experiment seen in segment 2 of the tracing is probably a very good expression of what is going on in a case of mitral stenosis, where there is a fair degree of cardiac competence but decompensation is close at hand. Under such circumstances the lung tissue is overfilled with blood; the alveoli are encroached upon by the swollen capillaries; lung movements are reduced since the highly elastic lung has been converted into a sort of erectile tissue.

Two fundamental effects may be expected from obstruction of the pulmonary veins; first, increased pressure in the lung capillaries, and second, decreased oxygenation of the blood due to interference with alveolar ventilation. Both of these favor leakage of plasma from the alveolar capillaries. The lung edema will mean increased tissue fluid in the alveolar walls with the possibility of leakage through the alveolar epithelium into the alveolar air spaces.

Figure 6 is a more complete representation of what occurred in the experiment under discussion and to a degree shown in figure 5. Lung lymph was collected for 3 hours and the amounts in milligrams per minute varied but little as seen in the second curve, *L.L.* At the beginning of the third hour the pulmonary veins were compressed for 10 minutes and then released. The effects of this obstruction upon systemic and pulmonary blood pressures are seen in figure 5, and are shown diagrammatically in figure 6, curve 3, *A.P.*, and curve 4, *P.A.P.*

In this animal, the pulmonary venous obstruction caused a sharp rise in lymph flow which, during the following hour and a quarter, fell to normal. With the increase in volume there went a fall in the per cent of protein in the lymph, which occurred in spite of the fact that in such experiments the increased pressure in the pulmonary capillaries invariably caused the lymph to appear bloody, but in the lungs as in other regions, escape of red cells from the capillaries frequently cannot be correlated with escape of plasma. For example, if the veins to a part, such as the foot of the dog, are slightly obstructed and the animal walks at a steady rate during the experiment, lymph flow from the foot becomes greater as the capillary pressure is raised and red cells, which during periods of normal venous pressure have been difficult to find in the lymph, soon become very

numerous. But with this increase in the apparent blood content of the lymph, the percentage of protein in it usually falls.

In the experiment described, a certain degree of anoxia accompanied the vascular congestion and possibly contributed a direct increase in the permeability of the capillary endothelium which made the heightened pressure in the pulmonary circuit even more effective in forcing fluid out of the capillaries and thus bringing about increased production and flow of lymph. In this case arterial oxygen was 18.2 cc. per 100 cc. of blood prior to vein compression and fell to 14.7 cc. per 100

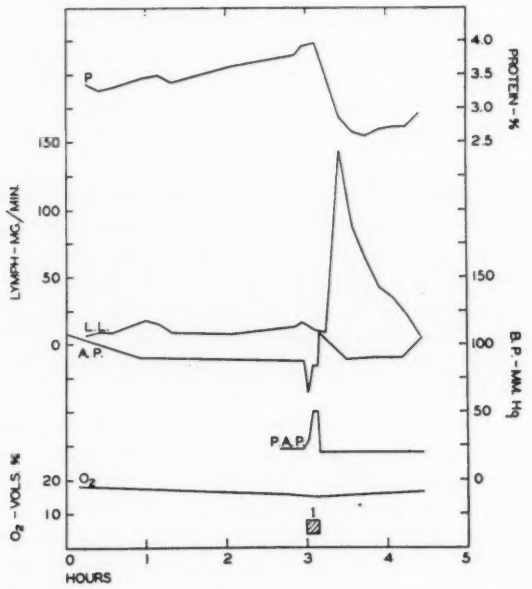

Fig. 6. Analysis of the effects of pulmonary vein compression. *Upper curve, P*, protein in lung lymph in per cent. *Second curve, L.L.*, milligrams of lymph per minute. *Third curve, A.P.*, pressure in the femoral artery in millimeters of mercury. *Fourth curve, P.A.P.*, pressure in the pulmonary artery in millimeters of mercury. *Fifth curve*, O_2, cubic centimeters of oxygen per 100 cc. of arterial blood. *Ordinates*, as designated. *Abscissae*, time in hours. During the period measured by the hatched square, *1*, the pulmonary veins were compressed.

cc. just before releasing the clamp and was 16.5 cc. at the close of the experiment when lymph flow had fallen to normal. This means definite but not pronounced oxygen lack. Corresponding figures for arterial carbon dioxide were 39.5, 38.3 and 39.7 cc. per 100 cc. of blood. These figures are quite normal and point to increased capillary pressure as the chief, if not the single cause of the augmented lymph flow. In another experiment, which resulted in an even larger flow of very bloody lymph, blood oxygen was 20.3 cc. per 100 cc. prior to pulmonary congestion, 17.9 cc. during the period when clamping was maintained, with a rise to 19.1 cc. 64 minutes after release. Figures for carbon dioxide content of the

same samples were 39.5, 35.3, 40.9 cc. per 100 cc. In this case lymph flow was still above the normal level when the experiment was ended and was extremely bloody.

C. *The path of drainage of lung lymph.* Using the method of exposure of the anterior mediastinum, which has been described, it is easy to clean the subclavian and adjacent veins with absolute thoroughness and thus to expose all possible entrances of the right lymphatic duct and the thoracic duct into the veins at the base of the neck. The thoracic and right ducts, or upon the right side, the efferent duct from the most cranial of the right tracheobronchial nodes were cannulated as they entered the veins.

These preparations made and lymph flow being normal, a glass catheter was passed down the trachea and into a right or left lower lobe bronchus, being pushed in gently until binding of the lower end precluded leakage. Three cubic centimeters of the dye T-1824 in physiological salt solution were then introduced into the catheter and when it was clear of fluid, so that overflow of the dye into the opposite lung would not occur, the catheter was withdrawn and artificial respiration, which had gone on unbrokenly in other parts of the lungs again involved the injected lobe. In some instances the dye was introduced in equal amounts into both lower lobes or into other lobes upon either side. The lymph from the thoracic duct and from the vessel cannulated upon the right side was watched for the first appearance of the intense blue dye and at the end of the experiment an autopsy was performed in order to follow the course of the stained lymphatics and nodes and to ascertain the distribution of the dye which had been given.

Table 3 shows the results of this series of experiments. Invariably the dye appeared first and in greater concentration on the right, regardless of where it had been placed. The medial tracheobronchial node was always deeply stained and the tracheobronchial nodes of the injected side were usually more heavily stained than those of the opposite side. In experiment 7 a trace of blue appeared in the thoracic duct two hours after its injection into the lung and an hour and a quarter after its first appearance in very concentrated amount in the right duct. Examination of mesenteric lymphatics at this time showed them to be slightly blue. The trace of dye in the thoracic duct had undoubtedly reached it, not from the lungs, but indirectly from the blood after absorption into the blood in the lungs, something that occurs in the case of T-1824, though in small amount.

In a number of experiments, usually when the dye had been placed in the left lung, either none or only a trace of dye could ever be detected in the thoracic duct. At autopsy a separate, blue-stained vessel of consequential size was sometimes found entering the junction of the jugular and subclavian veins on the left, quite independently of the thoracic duct. It has always been assumed, in spite of Sappey's work, that, by tying off the thoracic duct just above the diaphragm and cannulating it at its venous entrance, all lymph except from the heart and left lung would be excluded. The presence of this separate vessel, however, explains the fact that very often in our experience and in that of others, there is absolutely no flow from the cannulated duct under such circumstances.

It is also possible that in the dog somewhat the same situation exists as in man; i.e., that lymph from the left lower lobe, where the dye was placed, after entering the medial tracheobronchial node, which was always deeply stained, passed much more readily to the right tracheobronchial nodes than to the left. Under these circumstances the dye would naturally be found first and in greater concentration on the right, as was our invariable experience. The presence of connecting lymphatics between the nodes on the right and left of the trachea could account for the staining of the nodes and for the small amount of dye which was found later in the thoracic duct or in a separate vessel.

DISCUSSION. The experiments we have described have their chief value in the demonstration of a constant and considerable flow of lymph from the lungs, under conditions which are abnormal only in that the chest is open and artificial

TABLE 3

The appearance of dye in the final lymphatic path to the blood after intrabronchial injection into the lungs of the dog

NO. OF EXPERIMENT	LOCATION OF T-1824	FIRST APPEARANCE OF DYE	AMOUNT OF DYE						COMMENTS
			Right duct	Thoracic duct	Separate branch into veins on left	Medial tracheobronchial node	Right tracheobronchial nodes	Left tracheobronchial nodes	
1	Right and left lungs	Rt. duct	++++	−	+	++++	++++	++++	
2	Right and left lungs	Rt. duct	++++	++	−	++++	−	++++	
3	Right lung	Rt. duct	++++	−	−	++++	++++	−	
4	Right lung	Rt. duct	++++	−	−	++++	++++	++	
5	Right lung	Rt. duct	++++	−	−				Nodes not examined
6	Right lung	Rt. duct	++++	−	++	++++	++++	++++	
	Left lung	Rt. duct	++++	±	−	++++	+	++++	
	Left lung	Rt. duct	++++	±	++	++++	+	++++	Abdominal lymph blue
9	Left lung	Rt.+T. duct	++++	+++	+++	++++	+	++++	
10	Left lung	Rt. duct	++++	+	++	++++	++	+++	
11	Left lung	Rt. duct	++++	−	++	++++	++	+++	

respiration by means of positive pressure, replaces normal, negative pressure breathing. Reasons have been given for the belief that in the closed chest under the usual conditions of breathing, lymph flow is probably greater than under the circumstances of our experiment.

It has been shown that overventilation by positive pressure artificial respiration reduces lymph flow and that in the absence of respiratory movements the flow is also reduced. This last observation is of practical interest in relation to the condition of affairs in a lung rendered quiescent by pneumothorax. Where lymph drainage is interrupted, fibrosis occurs and this fact may well be of importance in the healing of infected areas in the lungs.

We have been able to report the effects of ventilating the lungs with mixtures low in oxygen and have attributed the increased lymph flow, which invariably

occurred, to increased permeability of the pulmonary capillaries. Against this assertion it may be held that no data upon cardiac output have been supplied. Granting the increased flow of lymph, the question arises as to whether this is not a simple expression of a larger, better circulated capillary bed which has occurred as a result of anoxia. It is our belief that this second explanation of augmented lymph flow is not correct. The reasons for this belief, or better, conviction, are as follows:

1. During the control period when ventilation with pure oxygen was used and throughout the subsequent low-oxygen ventilation, the movements of the lungs have been absolutely the same, curare being used to prevent spontaneous respiration. The anesthetized and curarized animal made no respiratory or other movements which could increase cardiac filling and thus induce greater cardiac output.

2. There is no evidence that the pulmonary capillaries open and close as active adaptations to circulatory requirements, their changes in diameter and in conduction of blood being passively controlled by cardiac output and probably also by respiratory movements.

3. The immediate effects of anoxia upon cardiac output are not reported to increase it, even in naturally breathing subjects (Grollman, 1932; Doi, 1921).

4. If the pulmonary capillaries lose tone as a result of asphyxia and the vascular bed becomes greater, the filtering pressure in the capillaries will fall unless the output of the right ventricle is increased. Simple mechanical conditions for enhanced filtration from pulmonary capillaries have not been provided by our experiment and the conclusion seems unescapable that the pulmonary capillaries are peculiarly susceptible to oxygen lack as a cause of increased permeability.

This conclusion is of importance clinically since edema of the lungs progressively excludes capillaries from access to the alveolar air and, therefore, a condition initiated by oxygen lack possesses capacity to decrease oxygen supply and thus a vicious circle is established. The lymphatics of the lungs counteract this course of events by steady removal of proteinized fluid and if, as is common, the pulmonary edema is accompanied by some increase in breathing, it is probable that lymph flow is greater than normal.

Another point relative to the experiments upon anoxia and those in which passive congestion of the lungs was produced by compression of the pulmonary veins, is the use which may be made of the volume and composition of the lymph to indicate the progress of pulmonary edema, or of increased fluid in the lungs from inflammation. The experimental analysis of such conditions has always suffered from inability to follow progressive changes, the autopsy and the histological section being our best objective evidence of the degree of edema, particularly in the early stages.

We have been able to give little attention to the effects of adequate oxygen in checking the progress of pulmonary edema. So far as has been seen the increased permeability caused by oxygen lack occurs quickly and is rapidly reversible if the process has not gone too far. The same general results upon lymph flow accompany experiments upon the heart and the cervical region in the dog.

Since the lung capillaries not only owe their oxygen supply to the alveolar air but are so placed that abnormal permeability upon their part due to anoxia induces further anoxia, it is impossible to utilize knowledge gained from other regions in arriving even at opinions in regard to recovery of normal permeability. It is, however, clear that administration of oxygen will become progressively less efficacious as pulmonary edema progresses. There is a "factor of safety" in all physiological conditions and at times it may prove deceptive. Thus the capillaries in a region of the lung where pulmonary edema has occurred may still possess a fair measure of normal resistance to leakage, but if adequate oxygenation is not provided, the unfavorable condition of the capillary walls may cause loss of the measure of normal permeability they still possess. If oxygen administration prevents this disastrous train of events, the earlier it can be given the better. Many questions arise in regard to the use of oxygen in combating pulmonary edema. For example: Is it essential that oxygen be given continuously, or will good results be obtained by periods of, let us say, 30 minutes' administration of oxygen, then 15 minutes of air or some such combination? If dyspnea is not marked, would the lungs clear more rapidly by inducing an increase in breathing through addition of carbon dioxide and so promoting increased lymph flow? If diffuse pulmonary inflammation is present, as, for example, in phosgene poisoning, how effective is oxygen or oxygen plus carbon dioxide in combating the effect? Certainly in such states anoxia adds to the effect of the poison in causing capillary leakage, but how far oxygen may be of immediate benefit to the injured tissue is not known.

In all of these problems studies of pulmonary lymph flow and composition may be useful and experiments are now in progress to supply this information.

SUMMARY

1. A method for collecting lymph from the lungs of the dog is described. The experiment requires opening the chest to expose the anterior mediastinum and artificial respiration is used during its course.

2. The composition of lung lymph resembles that of cardiac lymph. In 18 dogs the average protein content was 3.66 per cent.

3. When artificial respiration is unduly great or when the lungs are quiescent lymph flow is greatly reduced.

4. Ventilation with a mixture low in oxygen invariably increases lymph flow. If pressure in the pulmonary veins is heightened, lymph flow is greatly augmented and the lymph soon resembles blood.

5. The drainage of lymph from both lungs is in the main via the right lymphatic duct, comparatively little lung lymph being delivered to the circulation by the thoracic duct.

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